

CHAPTER

6

Bacteriology

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I. AEROBIC GRAM-POSITIVE BACTERIA

A. Staphylococci and Similar Microorganisms

1. *Staphylococcus aureus*

- a. Approximately 30% of the population carries *S. aureus* as resident flora, primarily in the anterior nares.
- b. Isolated from abscesses, wound infections, and carbuncles
- c. Causes food poisoning (via enterotoxin), pneumonia, osteomyelitis, endocarditis, wounds, staphylococcal scalded skin syndrome, etc.
- d. Produces six types of enterotoxin and **toxic shock syndrome toxin-1** (TSST-1)
- e. **Identifying characteristics**
 - 1) Gram-positive cocci arranged in clusters
 - 2) Colonies are opaque and smooth. *S. aureus* grows well on most media and is usually beta-hemolytic on sheep blood agar (SBA).
 - 3) Catalase and coagulase positive
 - 4) Latex agglutination assay detects clumping factor and protein A on the surface of *S. aureus*.
 - 5) Negative for ability to metabolize the substrate pyrrolidonyl- α -naphthylamide (**PYR**) and ornithine
 - 6) Staphylococci can tolerate the high salt concentration (7.5%) of **mannitol salt agar** (MSA).
 - a) *S. aureus* ferments mannitol and produces yellow colonies on MSA.
 - b) Most coagulase-negative staphylococci do not ferment mannitol and therefore produce red colonies.
 - 7) Penicillin resistance is due to **beta-lactamase** production. Methicillin-resistant *S. aureus* (**MRSA**) is resistant to β -lactam antibiotics because of production of altered penicillin-binding proteins. Rare strains of vancomycin-intermediate *S. aureus* (**VISA**) and vancomycin-resistant *S. aureus* (**VRSA**) have been reported. Vancomycin resistance is due to the VanA operon that alters the target of vancomycin in the cell wall. VISA occurs following overproduction of the target.

2. Coagulase-negative staphylococci

- a. Coagulase-negative staphylococci are very common skin flora and are mostly nonpathogenic. However, they can cause disease in immunosuppressed and neutropenic patients. This group of bacteria causes urinary tract infections (UTIs) and is associated with infections of catheters and shunts.
- b. Gram-positive cocci arranged in clusters
- c. Colonies appear white to gray on blood agar and nonhemolytic.
- d. Catalase positive and coagulase negative
- e. Commonly encountered species
 - 1) *Staphylococcus epidermidis*—Most common species of coagulase-negative staphylococci, novobiocin susceptible

- 2) *S. saprophyticus*—Significant only in UTIs, novobiocin resistant
 - 3) *S. lugdunensis*—Frequent cause of endocarditis, ferments manitol, PYR positive, and typically clumps in plasma (slide coagulase) because of the presence of clumping factor
3. **Micrococcus**
- a. *Micrococcus* spp. are **considered normal flora** of the skin and mucous membranes; they rarely cause infections.
 - b. On Gram stain, arranged in tetrads and appear larger than *Staphylococcus* spp., see Table 6-1 ■.
 - c. Colonies often appear yellow and nonhemolytic on SBA.

B. Streptococcaceae and Similar Microorganisms

1. General Characteristics

- a. Catalase-negative, gram-positive cocci arranged in pairs and chains
- b. Can be alpha- or beta-hemolytic, or nonhemolytic on SBA
- c. Lancefield grouping is based on a cell wall antigen.

2. Group A Streptococcus (*S. pyogenes*)

- a. Infections are spread by respiratory secretions, and some children may carry the bacteria in the respiratory tract without illness. However, *S. pyogenes* is always considered pathogenic.
- b. Infections caused include **strep throat** (pharyngitis), impetigo, cellulitis, scarlet fever, pneumonia, otitis media (middle ear infections), and **necrotizing fasciitis**.
- c. Sequelae include **rheumatic fever** and **post-streptococcal glomerulonephritis**.
- d. Susceptible to **bacitracin** (A disk) and PYR positive, but often identified by serological latex agglutination test
- e. Colonies are pinpoint (<1 mm), translucent, and will show a large zone of beta-hemolysis.
- f. Virulence factors include
 - 1) Cell wall **M protein** inhibits phagocytosis, and antibodies to M protein are protective.

TABLE 6-1 COMPARISON BETWEEN MICROCOCCI AND STAPHYLOCOCCI

	Micrococci	Staphylococci
Acid production from glucose under anaerobic conditions	O	+
Modified oxidase test	+	O
Bacitracin (0.04 unit disk)	S	R
O = negative, + = positive, R = resistant, S = sensitive		

- 2) **Streptococcal pyrogenic exotoxin** (Spe A, Spe B, Spe C, and Spe F), formerly referred to as **erythrogenic toxin**, causes the rash seen in scarlet fever. These toxins act as superantigens interacting with macrophages and T helper cells to stimulate the massive release of cytokines and are associated with streptococcal toxic shock syndrome (STSS).
 - 3) **Streptokinase** dissolves clots.
 - 4) **Hyaluronic acid** capsule inhibits phagocytosis.
 - 5) **Streptolysin O** and **streptolysin S** lyse erythrocytes, platelets, and neutrophils.
 - 6) Hyaluronidase hydrolyzes hyaluronic acid, an interstitial barrier, facilitating spread of the infection. Strains that produce a hyaluronic acid capsule will not produce hyaluronidase.
3. ***Streptococcus dysgalactiae* subsp. *equisimilis***
 - a. These isolates typically express **Lancefield group C or G antigens**.
 - b. The clinical spectrum of disease resembles *S. pyogenes* and includes pharyngitis, skin infections, necrotizing fasciitis, STSS, endocarditis, glomerulonephritis, and acute rheumatic fever.
 4. **Group B *Streptococcus* (*S. agalactiae*)**
 - a. **Normal flora** of the gastrointestinal tract of humans and animals
 - b. Important cause of infections in OB/GYN patients; 25% of all females carry the bacteria as normal vaginal flora
 - c. With **early onset infections**, neonates acquire infections during birth, resulting in sepsis and meningitis. Additionally, *S. agalactiae* can cause postpartum fever, osteomyelitis, and wound infections, as well as endocarditis, pneumonia, and pyelonephritis in immunosuppressed patients.
 - d. Colonies are medium-size (>1 mm), flat, creamy, and show small zones of beta-hemolysis. Some strains may be nonhemolytic.
 - e. **CAMP test and hippurate hydrolysis positive, PYR negative**
 - f. Unlike *S. pyogenes*, *S. agalactiae* is **resistant to bacitracin**. However, isolates are often identified by serological latex agglutination kits. Many other beta-hemolytic streptococci are also resistant to bacitracin.
 5. **Group D *Streptococcus***
 - a. **Normal fecal and oral flora**
 - b. These bacteria are associated with wound infections, UTIs, and abdominal abscesses. Isolation of group D streptococci in blood cultures is an indicator of colon cancer.
 - c. Colonies are gray to white, translucent, round, and convex.
 - d. Alpha-hemolytic or nonhemolytic, rarely beta-hemolytic
 - e. **Bile-esculin positive, negative for growth in 6.5% NaCl, PYR negative**
 6. **Viridans streptococci**
 - a. **Normal flora** of the oral cavity, respiratory tract, and gastrointestinal (GI) tract mucosa

- b. Major cause of bacterial **endocarditis** in people with damaged heart valves; also causes wound infections and brain abscesses
 - c. May enter the blood after dental procedures
 - d. Viridans *Streptococcus* spp. include *S. mutans* group, *S. salivarius* group, *S. sanguis* group, *S. bovis* group, and *S. mitis* group.
 - e. Alpha-hemolytic, some strains nonhemolytic
 - f. Optochin resistant and insoluble in bile; does not grow on bile-esculin medium
7. ***Streptococcus pneumoniae***
- a. **Normal upper respiratory tract flora but can cause:**
 - 1) **Lobar pneumonia** in the elderly and alcoholics
 - 2) **Otitis media** in infants and children
 - 3) **Meningitis**; however, a pediatric vaccine is available that has reduced the number of childhood meningitis cases
 - b. *S. pneumoniae* is an important cause of community-acquired bacterial pneumonia. Sputum samples are often rust-colored from blood.
 - c. Gram-positive diplococci that are **lancet or bullet shaped**, and alpha-hemolytic
 - d. Grows on SBA with 5–10% CO₂, at 48 hours
 - e. **Colony morphology**
 - 1) **Mucoid** strains produce a large polysaccharide capsule.
 - 2) **Umbilicated**, depressed centers caused by autolytic enzymes
 - 3) After 48 hours, colonies become nonviable.
 - f. **Optochin** (O or P disks) will inhibit growth (zone of inhibition), and *S. pneumoniae* is bile (10% sodium deoxycholate) soluble.
8. ***Enterococcus***
- a. Most commonly encountered species are *E. faecalis* and *E. faecium*.
 - b. **Identifying characteristics**
 - 1) **Bile-esculin positive**
 - 2) **Positive for growth in 6.5% NaCl**
 - 3) PYR positive
 - 4) Express Lancefield group D antigen
 - 5) Can be alpha-, beta-, or most commonly nonhemolytic
 - c. **Vancomycin-resistant enterococci (VRE):** Resistance is due to altered peptidoglycan cross-link target, D-Ala–D-Ala to D-Ala–D-Lac or D-Ala–D-Ser. The vast majority of VRE are *E. faecium*.
9. ***Gemella***
- a. *Gemella* spp. have been associated with a number of infections, including endocarditis, meningitis, brain abscesses, lung abscesses, and osteomyelitis.
 - b. PYR and leucine aminopeptidase (LAP) positive, and bile-esculin negative
10. ***Leuconostoc***
- a. *Leuconostoc* spp. have been linked to osteomyelitis, ventriculitis, postsurgical endophthalmitis, and bacteremia in neonates.

- b. The *Leuconostoc* spp. are vancomycin resistant, and PYR, LAP, and catalase negative.
- 11. ***Abiotrophia* and *Granulicatella***
 - a. Formerly referred to as **nutritionally variant streptococci**; require vitamin B₆ (pyridoxal or pyridoxamine) for growth
 - b. Species include *A. defectiva*, *G. adiacens*, and *G. elegans*.
 - c. These species are normal flora of the oral cavity and have been associated with endocarditis, ophthalmic infections, and infections of the central nervous system (CNS).

C. Aerobic Non-Spore-Forming Gram-Positive Bacilli

- 1. ***Listeria monocytogenes***
 - a. Causes spontaneous abortion and meningitis in animals (e.g., sheep)
 - b. Found in the environment (soil and water), and is normal flora of the vagina and intestines in humans
 - c. *L. monocytogenes* causes a variety of infections in neonates, pregnant women, and immunosuppressed patients. **Meningitis** is a common outcome of infection.
 - d. **Identifying characteristics**
 - 1) *L. monocytogenes* grows on most media; colonies are small and white with a narrow zone of beta-hemolysis.
 - 2) Closely resembles group B streptococci on SBA
 - 3) *L. monocytogenes* demonstrates both umbrella motility in semisolid media at room temperature and end-over-end (tumbling) motility in a wet mount.
 - 4) Hippurate hydrolysis, CAMP test, esculin, and catalase positive
- 2. ***Corynebacterium***
 - a. ***Corynebacterium diphtheriae*** causes diphtheria.
 - 1) Diphtheria is characterized by a pseudomembrane formed by dead cells and exudate at the back of the throat.
 - 2) Bacterial toxin damages major organs, resulting in a high death rate.
 - a) **Toxigenic *C. diphtheriae* strains** are infected with a bacteriophage that contains the gene for the diphtheria toxin.
 - b) Nontoxigenic *C. diphtheriae* strains lack the bacteriophage gene and do not produce the diphtheria toxin.
 - 3) Found only in humans
 - 4) **Identifying characteristics**
 - a) Gram stain: Diphtheroid morphology arranged in “picket fences” or “Chinese letters”; can be very pleomorphic
 - b) Staining with methylene blue will reveal metachromatic granules, which are red to purple intracellular granules.
 - c) Urease and pyrazidamidase negative, nitrate and catalase positive, and nonmotile

- d) The **Elek** test uses antitoxin to detect toxin production.
- e) Media
 - i. **Cystine-tellurite:** *Corynebacterium* spp. form black colonies from hydrolysis of tellurite.
 - ii. **Tinsdale's agar:** *Corynebacterium* spp. form brown to black colonies with halos from hydrolysis of tellurite.
 - iii. **Loeffler agar** is a nonselective medium that supports growth and enhances pleomorphism and the formation of metachromatic granules. Most *Corynebacterium* spp. produce small, white to gray colonies.
 - iv. *C. diphtheriae* will grow on SBA as small, white, dry colonies. Most strains are nonhemolytic.
- b. ***Corynebacterium jeikeium***
 - 1) *C. jeikeium* is an important cause of **nosocomial infections** and produces infections after prosthetic device implants and infections in immunocompromised patients.
 - 2) Pyrazidamidase positive
 - 3) Resistant to most antimicrobial agents
- c. ***Corynebacterium urealyticum***
 - 1) Cause UTIs
 - 2) Is rapid urease positive and grows very slowly
- 3. ***Arcanobacterium***
 - a. Six species of *Arcanobacterium* have been named; three are clinically significant: *A. haemolyticum*, *A. pyogenes*, and *A. bernardiae*.
 - b. The natural habitat of these organisms has not been confirmed, although *A. haemolyticum* has been associated with pharyngitis and wound and tissue infections. *A. pyogenes* is found on mucous membranes of cattle, sheep, and swine and is linked to abscess formation, wound, and soft tissue infections in cattle and humans.
 - c. The clinically significant *Arcanobacterium* spp. form small beta-hemolytic colonies on SBA and are catalase negative and nonmotile.
 - d. *A. haemolyticum* can be identified by the CAMP inhibition test. This bacterium produces phospholipase D, which inhibits the activity of the *Staphylococcus aureus* beta-lysin. *Corynebacterium pseudotuberculosis* also exhibits this phenomenon.
- 4. ***Erysipelothrix rhusiopathiae***
 - a. *E. rhusiopathiae* primarily infects animals. Humans generally become infected through contact with infected animals (occupational exposure) or rarely by consuming infected meat.
 - b. Human infections often result in cellulitis (erysipeloid lesions that can resemble erysipelas caused by *Streptococcus pyogenes*) but may also present as bacteremia or endocarditis.

- c. **Identifying characteristics**
 - 1) Nonmotile, pleomorphic gram-positive bacilli
 - 2) Catalase negative
 - 3) Hydrogen sulfide positive
- 5. *Nocardia asteroides*
 - a. Generally found in immunocompromised patients with chronic pulmonary disorders
 - b. *N. asteroides* is the most clinically relevant species; other species include *N. brasiliensis* and *N. otitidiscaviarum*.
 - c. **Identifying characteristics**
 - 1) **Pleomorphic, branching gram-positive bacilli** in chains that produce a beading arrangement, appear fungal-like
 - 2) **Partially acid-fast**, catalase positive, nonmotile
 - 3) Requires up to 6 weeks for growth
 - 4) Exudate contains masses of filamentous organisms with pus that resemble sulfur granules.

D. Aerobic Spore-Forming Gram-Positive Bacilli

- 1. **General Characteristics of *Bacillus* spp.**
 - a. **Bacterial spores** can survive adverse conditions for prolonged periods of time and are frequent contaminants of laboratory cultures.
 - b. Spores can be central or terminally located.
 - c. Most *Bacillus* spp. are nonpathogenic, and only genus identification is necessary.
 - d. *B. anthracis* (anthrax) and *B. cereus* (food poisoning and wounds) are pathogenic species.
 - e. *Bacillus* spp. form straight bacilli, with square ends (boxcar morphology) appearing in chains and singly.
 - f. The majority of the species will grow on SBA and phenylethyl alcohol (PEA) agar and are **catalase positive**.
 - g. Cultures form large, flat colonies.
- 2. *Bacillus anthracis*
 - a. Causes anthrax, a zoonosis that is rare in the U.S.
 - b. Three clinical forms of anthrax
 - 1) **Cutaneous anthrax:** Most common form worldwide, characterized by necrotic skin lesions called black eschars
 - 2) **Pulmonary anthrax:** “Wool-sorter’s disease,” spread by inhalation of spores from sheep’s wool
 - 3) **Gastrointestinal anthrax:** Rarest form; follows ingestion of spores
 - c. *B. anthracis* is considered a **potential bioterrorism agent** and was used as such in a series of attacks in the U.S. in 2001.
 - d. It produces large, **nonhemolytic colonies** with filamentous projections, sometimes referred to as “Medusa-head” colonies.

- e. *B. anthracis* typically does not grow on PEA agar at 24 hours.
 - f. **Preliminary testing by sentinel laboratories:** Typical colony morphology, gram-positive bacilli with spores, catalase positive, and nonmotile
 - g. Confirmatory testing is performed by a reference laboratory.
3. *Bacillus cereus*
- a. *B. cereus* is an important cause of **food poisoning** and occasionally wounds. It may also cause opportunistic eye, bone, and brain infections.
 - b. *B. cereus* and *B. subtilis* are also common **laboratory contaminants**.
 - c. Colony morphology: Large, flat, beta-hemolytic colonies with irregular edges
 - d. **Motile** and resistant to 10 µg of penicillin

II. AEROBIC GRAM-NEGATIVE BACTERIA

A. *Neisseria* and Similar Microorganisms

1. Family *Neisseriaceae*

- a. Includes the genera *Neisseria*, *Eikenella*, *Kingella*, and *Simmonsella*
- b. Many species are normal flora of the upper respiratory tract of animals.
- c. Kidney bean-shaped, gram-negative diplococci or coccobacilli
- d. The *Neisseriaceae* are oxidase positive, fastidious, and grow best in 5–10% CO₂ at 37°C. They cannot tolerate cold; therefore, media must be at room temperature before plating.

2. *Neisseria gonorrhoeae*

- a. Humans are the only host for *N. gonorrhoeae*. It is fastidious and does not survive long outside the host. *N. gonorrhoeae* can be isolated from the urethra, cervix, anal canal, oropharynx, skin lesions, joints, and blood.
 - 1) **In males** it causes acute urethritis, which is characterized by a pus-containing urethral discharge and dysuria and can also cause prostatitis and epididymitis.
 - 2) **In females** it causes urethral infections and cervicitis. Infections can be asymptomatic or produce cervical discharge, fever, acute pain, and dysuria. *N. gonorrhoeae* can also cause pelvic inflammatory disease (PID), gonococcal arthritis, salpingitis, endometritis, and peritonitis.
- b. **Neonates** may be infected during vaginal delivery, resulting in gonococcal ophthalmia neonatorum, which is a severe conjunctivitis leading to blindness. To prevent newborn conjunctivitis, antimicrobial eye drops (e.g., erythromycin) are administered to all infants at birth.
- c. *N. gonorrhoeae* is not normal flora.
- d. On direct Gram stain, *N. gonorrhoeae* often appears **intracellular in neutrophils**.
- e. Must culture immediately, and clinical material must be free of lubricants and spermicides
 - 1) *N. gonorrhoeae* is fastidious, requiring enriched media such as chocolate. **It does not grow on SBA.**

- 2) **Selective media** include modified Thayer-Martin, Martin-Lewis, New York City, and GC-Lect agars.
- 3) The bacteria require increased CO₂ with a humidified atmosphere.
- 4) Because of autolysis, gonococci cannot be incubated for prolonged times.
- f. Colonies are flat, smooth, and glistening gray or tan.
- g. **Identifying characteristics**
 - 1) Superoxol, catalase, oxidase, and glucose positive
 - 2) Maltose, lactose, sucrose, DNase, and nitrate negative
- h. Many strains are positive for beta-lactamase production.
3. ***Neisseria meningitidis***
 - a. Spread by respiratory droplets and may be normal flora of the nasopharynx
 - b. Causes meningococcal meningitis, meningococcemia, leading to disseminated intravascular coagulation, and Waterhouse-Friderichsen syndrome
 - c. Specimens: Cerebrospinal fluid (CSF), sputum, blood, and nasopharyngeal swabs
 - d. Colonies are flat, smooth, and gray to white on chocolate agar.
N. meningitidis will grow on SBA incubated in increased CO₂ and produce bluish-gray colonies.
 - e. **Identifying characteristics**
 - 1) Catalase, oxidase, glucose, and maltose positive
 - 2) DNase and nitrate negative
4. **Normal flora *Neisseria***
 - a. Many *Neisseria* spp. are normal flora of the upper respiratory tract. Species include *N. elongata*, *N. mucosa*, *N. lactamica*, *N. cinerea*, *N. polysaccharea*, *N. flavescens*, *N. subflava*, and *N. sicca*.
 - b. In rare cases, these organisms can cause meningitis, endocarditis, and other infections.
 - c. Many species are not fastidious and will grow on most nutrient agars.
5. ***Kingella***
 - a. *K. kingae* colonizes the throat of children, whereas the habitat of *K. denitrificans* is unknown. *K. oralis* has been isolated from the mouth of adults.
 - b. *K. kingae* shows a predilection for infections of the bones and joints in children. Infections in adults are generally limited to those who are immunocompromised. *K. denitrificans* is most often associated with endocarditis.
 - c. *K. kingae* is best isolated from joints and bones in blood culture media. *Kingella* will grow on sheep blood, chocolate, and modified Thayer-Martin agars. They will not grow on MacConkey agar.
 - d. The ability of *K. denitrificans* to reduce nitrates is a key test for its differentiation from *N. gonorrhoeae*.
6. ***Moraxella catarrhalis***
 - a. Member of the family *Moraxellaceae*
 - b. Resembles *Neisseria* and is normal flora of the upper respiratory tract

- c. Causes otitis media, sinusitis, and respiratory tract infections
- d. Will grow on most nutrient agars
- e. **Identifying characteristics**
 - 1) Catalase, oxidase, DNase, nitrate, and butyrate esterase positive
 - 2) Asaccharolytic; all carbohydrate tests are negative

B. *Enterobacteriaceae*

1. General family characteristics

- a. Most medically important family of gram-negative bacilli
- b. Most species are normal flora of the GI tract. *Salmonella*, *Shigella*, and *Yersinia* are not normal GI flora.
- c. Major cause of nosocomial infections
- d. Diseases include UTIs, gastroenteritis, septicemia, food poisoning, wound infections, peritonitis, pneumonia, and meningitis
- e. The family exhibits four **serological characteristics**
 - 1) **O (somatic) antigen**—A cell wall antigen (heat stable)
 - 2) **K (envelope) antigen**—Capsular antigen (heat labile)
 - 3) **H (flagellar) antigen**—Flagellar antigen (heat labile)
 - 4) **Vi antigen**—Capsular antigen of *Salmonella* Typhi (heat labile)
- f. *Enterobacteriaceae* are facultative anaerobes. They ferment glucose, are nitrate and catalase positive and, with the exception of *Plesiomonas*, are oxidase negative.

g. Enteric media

- 1) **MacConkey (MAC) agar:** Lactose-positive colonies are pink/red, and lactose-negative colonies are colorless.
- 2) **Eosin-methylene blue (EMB) agar:** Colonies of lactose fermenters have a dark center, and lactose nonfermenters are colorless. *E. coli* has a dark center and usually shows a green metallic sheen.
- 3) **Hektoen enteric (HE) agar:** Lactose and/or sucrose fermenters form yellow/orange colonies. *Salmonella* colonies are green with black centers (H_2S positive), and *Shigella* colonies are green.
- 4) **Xylose-lysine-desoxycholate (XLD) agar:** Colonies of lactose and/or sucrose fermenters are yellow. *Salmonella* produce red colonies with black centers (H_2S), and *Shigella* have clear colonies.
- 5) **Salmonella-Shigella (SS) agar:** Lactose fermenters produce red colonies; *Salmonella* colonies are colorless with black centers, and *Shigella* colonies are colorless.
- 6) **Bismuth sulfite agar:** *Salmonella* Typhi produces black colonies; lactose-fermenting colonies are yellow-orange.
- 7) **Brilliant green agar:** *Proteus* and *Salmonella* species produce red/pink colonies, whereas *Shigella* and most lactose fermenters will not grow.
- 8) **Selenite broth:** The broth is an enhancement medium for stool cultures. *Salmonella* growth is enhanced, whereas gram-positive and

coliform (normal GI flora) bacteria are inhibited. It is no longer commonly used.

2. Important genera

a. *Escherichia*

- 1) ***E. coli* is normal GI flora** and a very common clinical isolate.
- 2) *E. coli* causes UTIs, appendicitis, peritonitis, gallbladder infections, endocarditis, meningitis in newborns, gastroenteritis, and food poisoning.
- 3) **Identifying characteristics**
 - a) **Triple sugar iron (TSI):** Acid over acid (A/A) and H₂S negative
 - b) **MacConkey agar:** Pink/red colonies
 - c) On **SBA**, colonies are shiny, opaque, off-white, 2–4 mm in diameter, and usually **beta-hemolytic**.
 - d) **EMB agar:** Green metallic sheen colonies with dark centers
 - e) **Indole**, methyl red (MR), motility, and *o*-nitrophenyl- β -D-galactopyranoside (ONPG) positive
 - f) Voges-Proskauer (VP), citrate, and urease negative
- 4) **Enterohemorrhagic *E. coli* (EHEC)** causes hemorrhagic colitis and hemolytic uremic syndrome (HUS), leading to kidney failure in young children.
 - a) Acquired by eating undercooked hamburger or other contaminated foods such as apple cider, basil, sprouts, etc.
 - b) The principal virulence factor is Shiga toxin (Stx) 1 and 2. Many strains of Stx-producing *E. coli* belong to the serogroup O157:H7. However, several other serogroups have been reported that produce Stx-1 and/or Stx-2. *E. coli* O157:H7 is thought to cause over 80% of all cases of HUS in the U.S.
 - c) Growth on **sorbitol-MacConkey (SMAC) agar:** Sorbitol replaces lactose in the medium. *E. coli* O157:H7 does not metabolize sorbitol; most other *E. coli* strains rapidly ferment sorbitol, producing pink colonies on SMAC. *E. coli* O157:H7 colonies appear colorless on SMAC. Confirmatory testing is by detection of Stx-1 and Stx-2.
- 5) Other strains of *E. coli* causing human intestinal infections
 - a) **Enterotoxigenic *E. coli* (ETEC)** produces severe epidemic diarrhea, mainly from drinking contaminated water.
 - b) **Enteroinvasive *E. coli* (EIEC)** causes bloody diarrhea by invading the intestinal epithelium.
 - c) **Enteropathogenic *E. coli* (EPEC)** causes a watery diarrhea.

b. *Shigella*

- 1) Causes shigellosis, a form of bacterial dysentery, characterized by abdominal pain, fever, and diarrhea
- 2) Infections are most severe in children and the elderly. Outbreaks are known to occur in daycare centers and nursing homes.

- 3) Highly pathogenic; Less than 50 bacteria can cause disease
- 4) Causes food poisoning by direct fecal contamination from infected humans
- 5) Incubation period is between 1 and 7 days.
- 6) **Four serogroups based on O antigens**
 - a) *S. dysenteriae* (serogroup A) produces an enterotoxin, which affects the large intestines, and a neurotoxin that may result in paralysis. *S. dysenteriae* is mannitol and ONPG negative.
 - b) *S. flexneri* (serogroup B) produces a mild diarrhea. It is mannitol positive and ONPG negative.
 - c) *S. boydii* (serogroup C) produces a mild diarrhea. *S. boydii* is mannitol positive and ONPG negative. It is difficult to biochemically distinguish *S. flexneri* from *S. boydii*.
 - d) *S. sonnei* (serogroup D) produces a mild diarrhea. It is the most common cause of shigellosis in the U.S. *S. sonnei* is mannitol and ONPG positive. It is a delayed lactose fermenter.
- 7) **Identifying characteristics**
 - a) TSI: Alkaline over acid (K/A)
 - b) H₂S, VP, motility, citrate, urease, and lactose negative
 - c) MR positive
- c. ***Klebsiella***
 - 1) *Klebsiella* spp. typically cause UTIs and pneumonia. Many infections are nosocomial, and diabetics and alcoholics are prone to infections.
 - 2) The most common species isolated is *K. pneumoniae*.
 - 3) **Identifying characteristics**
 - a) **TSI:** A/A with gas
 - b) On MAC agar, the pink colonies are very mucoid because of capsule production.
 - c) *Klebsiella* spp. are H₂S and MR negative and nonmotile. Except for *K. oxytoca* and *K. ornithinolytica*, the *Klebsiella* are indole negative.
 - d) VP, citrate, and lactose positive
 - 4) ***Klebsiella* (*Calymmatobacterium*) *granulomatis***
 - a) The causative agent of **granuloma inguinale**, a sexually transmitted disease
 - b) **Identification**
 - i. Does not Gram stain or grow on laboratory media
 - ii. In clinical specimens Wright or Giemsa stained, Donovan bodies may be seen. Donovan bodies are intracellular pleomorphic bipolar staining bacterial cells.
- d. ***Enterobacter***
 - 1) The genus includes about 12 species. They are found in soil, water, and dairy products.

- 2) *E. cloacae* is the most common, and *E. aerogenes* is the second most common species isolated. *Enterobacter* spp. are occasional clinical isolates that have been linked to respiratory tract infections and wounds and isolated from blood.
- 3) **Identifying characteristics**
 - a) H₂S, MR, and indole negative
 - b) VP and citrate positive
 - c) All species except *E. taylorae* are lactose positive.
 - d) *E. aerogenes* is arginine negative and lysine positive. *E. cloacae* is arginine positive and lysine negative.
 - e) *E. sakazakii* produces a yellow pigment that aids in its presumptive identification.
- e. ***Serratia***
 - 1) Causes opportunistic infections in patients undergoing chemotherapy and immunosuppressed patients
 - 2) ***S. marcescens* is the most common clinical isolate.**
 - 3) **Identifying characteristics**
 - a) DNase, gelatinase, and lipase positive, unique among the enterics
 - b) VP and citrate positive
 - c) ONPG positive but a delayed lactose fermenter
 - d) Some strains produce a red pigment, which is enhanced with room temperature incubation.
- f. ***Salmonella***
 - 1) **The genus now contains two species, *S. enterica* and *S. bongori*,** with over 2400 serotypes. Most serotypes are pathogenic to humans and cause moderate to severe gastroenteritis. The majority of human cases of salmonellosis are due to serotypes belonging to the species *S. enterica*, which includes the serotype *S. Typhi*.
 - 2) **There are many animal reservoirs.**
 - 3) Transmitted through contaminated water and undercooked food, especially chicken
 - 4) *Salmonella* Typhi causes **typhoid fever**, the most severe form of salmonellosis, which is characterized by a septicemia followed by a GI tract infection. Humans are the only reservoir for *S. Typhi*.
 - 5) Most human infections in the U.S. are caused by serotypes *Salmonella* Enteritidis and *Salmonella* Typhimurium.
 - 6) *Salmonella* isolated from stool cultures form lactose-negative and H₂S-positive colonies on enteric media.
 - 7) **Identifying characteristics**
 - a) H₂S, motility, and citrate positive
 - b) Indole, urease, and lactose negative
 - c) Colonies on HE agar are green with black centers.

g. *Proteus*

- 1) Four species are recognized. *Proteus vulgaris* and *P. mirabilis* are the **most common** isolates.
- 2) **Identifying characteristics**
 - a) *P. vulgaris* and *P. mirabilis* are typically H₂S positive.
 - b) *P. mirabilis* and many strains of *P. vulgaris* exhibit swarming motility on SBA.
 - c) All species are urease, tryptophan deaminase (TDA), and phenylalanine deaminase (PDA) positive.
 - d) ONPG and therefore lactose negative
 - e) *P. mirabilis* is indole negative; *P. vulgaris* is indole positive.

h. *Yersinia*

- 1) Three pathogenic species of *Yersinia*
 - a) ***Y. pestis* causes plague;** it is endemic to the southwestern U.S. Small animals (e.g., rodents) are natural reservoirs, and the bacteria are transmitted by fleas. *Y. pestis* is considered a potential bioterrorism agent.
 - b) ***Y. enterocolitica* causes enterocolitis** in humans; it is acquired by drinking contaminated water or by eating contaminated meat. Isolates are ONPG positive but delayed lactose fermenter and sucrose positive. Therefore, colorless on MAC at 18 hours, but A/A on TSI.
 - c) ***Y. pseudotuberculosis* is a rare cause of lymphadenitis in children.**
- 2) Small coccobacilli
- 3) **Cefsulodin-irgasan-novobiocin (CIN) medium** is a selective and differential medium for isolation of *Y. enterocolitica*. Colonies of *Yersinia* will ferment mannitol and absorb the dye, neutral red, producing clear colonies with a pink center. *Aeromonas* spp. will also grow on this medium and form colonies with pink centers.
- 4) All species except *Y. pestis* are nonmotile at 37°C but motile at 25°C. *Y. pestis* is nonmotile at both temperatures.
- 5) Presumptive identification of *Y. pestis* is based on isolation of the bacterium from respiratory tract, blood, or lymph nodes with the following characteristics: pinpoint colonies on SBA after 24 hours incubation, gram-negative bacilli, oxidase and urease negative, and catalase positive. Growth may be better at 28°C than 35°C. Confirmatory testing is performed by a regional reference laboratory.

i. *Edwardsiella*

- 1) *E. tarda* is the most common isolate.
- 2) **Resembles *Salmonella*,** H₂S positive, and ONPG negative
- 3) Unlike *Salmonella*, *E. tarda* is indole positive and citrate negative.

j. *Citrobacter*

- 1) *C. freundii* is the most common species isolated.
- 2) *C. freundii* resembles *E. coli* on MAC but can be differentiated because of being H₂S (+) and indole (–).

k. *Morganella*

- 1) *M. morganii* is the only species.
- 2) Indole positive VP and citrate negative
- 3) PDA and TDA positive

l. *Providencia*

- 1) There are five species of *Providencia*, and four have been isolated from humans. The most common isolate is probably *P. rettgeri*.
- 2) PDA, TDA, indole, and citrate positive and VP negative

C. *Haemophilis* and Similar Organisms

1. General characteristics

- a. Most species are normal upper respiratory tract flora.
- b. *Haemophilus* spp., especially *H. aphrophilus*, are considered members of the **HACEK** (*Haemophilus*, *Actinobacillus actinomycetemcomitans*, *Cardiobacterium hominis*, *Eikenella corrodens*, and *Kingella*) group. Members of this group are fastidious (i.e., require complex nutrients for growth) and important causes of endocarditis.
- c. Pleomorphic gram-negative coccobacilli ranging from very small to filamentous
- d. **Growth requirements** include hemin (X factor), which is released from hemoglobin, and/or NAD (V factor), which is a heat-labile compound, see Table 6-2■.
- e. *Haemophilus* spp. do not grow on SBA because of NADase in the agar (NADase inactivates NAD) but will grow on horse or rabbit blood agar, which contains no NADase. Chocolate agar is routinely used for cultures.
- f. Grows at 35–37°C with 5–10% CO₂ and is susceptible to drying and temperature changes
- g. Colony morphology: Smooth, round, flat, opaque, and tan on chocolate agar
- h. **Satellitism:** *Haemophilus* spp. can grow around colonies of *S. aureus* growing on an SBA plate. *S. aureus* releases NAD. Therefore, *Haemophilus* will grow near the *S. aureus* colonies, forming tiny clear pinpoint colonies.
- i. Nonmotile, catalase and oxidase positive

2. Clinically relevant species

a. *Haemophilus influenzae*

- 1) **Six capsular serotypes, a–f, and eight biotypes, I–VIII**
- 2) *H. influenzae* type b was a major cause of meningitis in children. The widespread use of the *Haemophilus influenzae* type b (Hib) **vaccine** has greatly reduced childhood meningitis and other invasive diseases caused by this serotype.

TABLE 6-2 IDENTIFICATION OF *HAEMOPHILUS* SPP

	Requires		β -Hemolysis on	ALA*
	X Factor	V Factor	Horse Blood	
<i>H. influenzae</i>	+	+	—	—
<i>H. ducreyi</i>	+	—	—	—
<i>H. aegyptius</i>	+	+	—	—
<i>H. aphrophilus</i>	—	—	—	+
<i>H. haemolyticus</i>	+	+	+	—
<i>H. parainfluenzae</i>	—	+	—	+
<i>H. paraphrophilus</i>	—	+	—	+

*Aminolevulinic acid (ALA) is converted to porphyrin, + = positive, — = negative

- 3) Serotypes other than b are frequent cause of respiratory tract infections, including acute sinusitis, chronic bronchitis, and pneumonia. Otitis media with effusion (middle ear infections) and sinusitis are often caused by nontypeable strains, those lacking a capsule.
- 4) ***H. influenzae* isolates should be tested for beta-lactamase.**
- 5) This species can be isolated from a variety of specimens, including blood, sputum, CSF, and eye swabs.
- 6) Specific detection of Hib capsular antigen is by latex agglutination.
- b. ***Haemophilus aegyptius***
 - 1) **Causes pink eye, a very contagious conjunctivitis**
 - 2) Similar to *H. influenzae* with the exception of being sucrose positive
- c. ***Haemophilus influenzae* biogroup aegyptius**
 - 1) Causes a conjunctivitis, followed by invasive disease known as Brazilian purpuric fever
 - 2) It resembles *H. influenzae* biotype III in that it is indole negative, urease positive, and ornithine decarboxylase negative.
- d. ***Haemophilus ducreyi***
 - 1) **Causes genital ulcers, a sexually transmitted disease**
 - 2) Produces chancroids and buboes (swollen lymph nodes)
 - 3) Chocolate agar with vancomycin is used to inhibit normal flora and contaminants.

D. Nonfermentative Gram-Negative Bacilli

1. General characteristics

- a. Found in water, soil, food, and plants, and a few are normal flora of humans
- b. Approximately 20% of all gram-negative bacilli isolates are nonfermentative gram-negative bacilli (NFB).
- c. **General characteristics of NFB**
 - 1) Most species are obligate aerobes.
 - 2) They do not form spores and do not metabolize carbohydrates under anaerobic conditions (fermentation).
 - 3) Most are oxidase positive.
 - 4) **TSI:** K/no change
 - 5) Grow on SBA but varied growth on MAC

2. *Pseudomonas aeruginosa*

- a. ***P. aeruginosa* is the most important NFB.** It is a common clinical isolate that can infect humans, animals, plants, and fish.
- b. *P. aeruginosa* is a member of the ***Pseudomonas* fluorescent group** along with *P. fluoresceins* and *P. putida*. All members of the fluorescent group produce **fluorescein (pyoverdin)**, a yellow pigment that fluoresces. Growth on **cetrimide agar** enhances fluorescein production.
- c. It causes eye (in contact lens wearers) and ear infections and is responsible for “swimmer’s ear,” which is an external otitis.
- d. Lower respiratory tract infections in patients with **cystic fibrosis (CF)**
- e. Causes burn wound infections
- f. Important pathogen in immunocompromised individuals
- g. *P. aeruginosa* is resistant to a number of disinfectants and has been responsible for serious **nosocomial infections**. It is especially associated with hospital environments and equipment, whirlpools, and swimming pools.
- h. **Identifying characteristics**
 - 1) Oxidase positive
 - 2) Motile
 - 3) Lactose negative
 - 4) **Colony morphology**
 - a) Large, irregular colonies with a grapelike odor and metallic sheen on SBA
 - b) **β -hemolytic** colonies with a feathery edge on SBA
 - c) **Mucoid colonies when isolated from patients with CF**
 - d) **Pigment:** Only *P. aeruginosa* produces **pyocyanin**, a blue pigment. Pyocyanin mixes with fluorescein to produce a blue-green color.
 - 5) **Oxidative-fermentative glucose test:** This test determines if glucose is broken down oxidatively or fermentatively. Two tubes are used; one is overlaid with mineral oil to produce anaerobic conditions. The other tube contains no mineral oil (aerobic). **Oxidative bacteria**

produce acid (yellow color) in the open tube, whereas the closed tube is green. **Oxidative/fermentative bacteria** produce acid in both tubes.

- i. **Very resistant to antimicrobial agents**
3. ***Stenotrophomonas maltophilia***
 - a. Acquired as transient flora from hospitals
 - b. Causes pneumonia, UTIs, wound infections
 - c. **Identifying characteristics**
 - 1) Oxidase negative
 - 2) *S. maltophilia* is one of the only nonfermentative, gram-negative bacillus that is oxidase negative and maltose positive
 - 3) One of two clinically encountered NFB that is lysine decarboxylase positive.
 - 4) Resistant to most antimicrobials
4. ***Burkholderia cepacia***
 - a. *B. cepacia* causes nosocomial infections and is also an important respiratory tract pathogen in patients with CF; second most common cause to *P. aeruginosa*.
 - b. Enhanced growth on ***P. cepacia* (PC) agar** that inhibits *P. aeruginosa*
 - c. Colony morphology: Colorless or yellow on nutrient agar
 - d. Oxidase and lactose positive
 - e. It is one of two clinically encountered NFB that is lysine decarboxylase positive.
5. ***Burkholderia mallei***
 - a. Causes **glanders**, a highly contagious disease of livestock, particularly among horses, mules, and donkeys
 - b. *B. mallei* can be transmitted to humans by animal contact. The bacterium is also considered a **potential bioterrorism agent**.
 - c. *B. mallei* grows on MacConkey agar, is oxidase variable and **nonmotile**, reduces nitrate to nitrite without gas, and oxidizes glucose.
6. ***Burkholderia pseudomallei***
 - a. Causes **melioidosis**, a disease of humans and animals endemic to Southeast Asia and northern Australia
 - b. *B. pseudomallei* is found in soil, and infections are acquired through the skin or by inhalation.
 - c. *B. pseudomallei* grows on MacConkey agar, is oxidase positive and **motile**, reduces nitrate to nitrite with gas, and oxidizes several sugars, including glucose and lactose.
7. ***Acinetobacter***
 - a. Obligate aerobic, coccobacillus found as normal flora of the GI and respiratory tracts
 - b. *Acinetobacter* spp. are important causes of nosocomial infections (most often *A. baumannii*) and UTIs. **Isolates tend to be multidrug resistant.**

- c. *Acinetobacter* spp. grow on most media and may resemble enterics on MAC and EMB agars. Some species, such as *A. baumannii*, produce acid from glucose (saccharolytic group), whereas others are asaccharolytic.
- d. **Identifying characteristics**
 - 1) Nonmotile and oxidase negative
 - 2) Nitrate negative and catalase positive

E. Miscellaneous Gram-Negative Bacilli

1. *Francisella*

- a. *F. tularensis* causes **tularemia** and is a **potential agent of bioterrorism**.
- b. **The bacteria are carried by wild animals**, including deer, rabbits, beavers, and squirrels.
- c. Humans may acquire the infection by skinning animals or eating undercooked game, or from animal bites and the bite of deerflies or ticks.
- d. Intracellular bacteria that resist phagocytosis
- e. *F. tularensis* causes skin ulcers at the site of inoculation and can cause infections of the lymph nodes, eyes, lungs, and GI system.
- f. Biosafety level 3 is required when handling the organism or suspect specimens.
- g. **Identifying characteristics**
 - 1) Faintly staining coccobacilli
 - 2) The medium of choice is glucose-cystine blood agar.
 - 3) Colony morphology: Small and grayish
 - 4) Agglutination and direct fluorescent antibody tests are used to confirm the identification.

2. *Brucella*

- a. **Causes brucellosis, also known as undulant fever**
- b. Normal gastrointestinal flora of animals
- c. Humans usually acquire the infection by drinking contaminated milk or from slaughter house exposure. The incubation period is 1–3 weeks.
- d. Four species infect humans: *B. melitenis*, *B. abortus*, *B. suis*, and *B. canis*. *B. melitenis* causes the most severe infections. *Brucella* has been considered a **potential bioterrorism agent**.
- e. Facultative intracellular parasite
- f. Biosafety level 3 organism
- g. Isolated from blood and **bone marrow**
- h. **Identifying characteristics**
 - 1) They are fastidious organisms but will grow on Brucella, buffered charcoal yeast extract (BCYE), and modified Thayer-Martin agars, and require 10% CO₂ in humidified air and 3–4 weeks for growth. Isolation in automated blood culture monitoring systems is recommended.
 - 2) Strict aerobe
 - 3) Oxidase and catalase positive

- 4) Serology can be used for presumptive diagnosis of brucellosis. Cultures are needed for definitive identification. Confirmation of identification of isolates as *Brucella* sp. is generally done serologically. Phage and dye sensitivity tests are used for identification to the species level.

3. *Bordetella*

- a. ***B. pertussis* causes pertussis.**
- b. It inhabits the mucous membranes of the respiratory tract of humans.
- c. Three stages of pertussis (whooping cough)
 - 1) **Catarrhal:** General flulike symptoms
 - 2) **Paroxysmal:** Repetitive coughing episodes
 - 3) **Convalescent:** Recovery phase
- d. ***B. pertussis* grows on Bordet-Gengou (potato infusion) and Regan-Lowe agars (charcoal-horse blood agar). Media are often made selective by adding cephalixin.**
- e. Other species
 - 1) ***B. parapertussis* causes mild respiratory infections in humans.**
 - 2) ***B. bronchiseptica* causes kennel cough in dogs and is an infrequent cause of respiratory infections in humans.**
- f. **Identifying characteristics**
 - 1) ***B. pertussis* colonies are small and smooth; they appear like mercury droplets and are beta-hemolytic.**
 - 2) **Gram stain shows minute, poorly stained coccobacilli, single or in pairs.**
 - 3) **Most species will grow on MAC agar except *B. pertussis*.**
 - 4) ***B. pertussis* is urease negative, whereas all other species are urease positive.**

4. *Actinobacillus*

- a. ***Actinobacillus* spp. are found mostly as oral flora of animals.**
A. actinomycetemcomitans is normal oral flora of humans.
- b. **Infections are caused by animal bites, which can result in cellulitis.**
A. actinomycetemcomitans is associated with endocarditis and also causes gum disease.
- c. ***Actinobacillus* spp. grow well on SBA and chocolate agar but will not grow on MacConkey agar. They produce colonies that show starlike centers.**
- d. **Most species are catalase and glucose positive.**

5. *Pasteurella*

- a. ***Pasteurella* spp. are normal respiratory/GI flora of animals. Humans acquire the bacteria from animal bites (cats and dogs) or by inhalation of dried animal feces.**
- b. **Causes cellulitis but can progress into osteomyelitis, meningitis, joint infections, and pneumonia**
- c. ***P. multocida* causes most human infections.**

- d. **Identifying characteristics**
 - 1) Grows well on nonselective agars but not MAC
 - 2) Oxidase, catalase, indole, and nitrate positive
 - 3) Nonmotile, pleomorphic, gram-negative coccobacilli that may show bipolar staining
 - 4) Very susceptible to penicillin
6. *Eikenella corrodens*
 - a. **Normal flora of the mouth and upper respiratory tract**
 - b. Causes abscesses of oral cavity and human bite wound infections
 - c. Approximately 50% of the strains **corrode or pit the agar surface**.
 - d. Requires hemin (factor X) for growth, unless 5–10% CO₂ is present
 - e. Produces a bleachlike odor
7. *Legionella*
 - a. First discovered in 1976 as the cause of pneumonia in people attending an American Legion convention in Philadelphia
 - b. *Legionella* spp. are aquatic organisms that may be found in various water systems, including humidifiers, whirlpools, and air conditioning chillers. They are resistant to commonly used concentrations of chlorine.
 - c. **Most human infections are caused by *L. pneumophila* serogroup 1.**
 - d. Causes **legionellosis**, which can be asymptomatic or mild to severe pneumonia. Legionnaires disease, a primary pneumonia, is the severe form of legionellosis. **Pontiac fever** is a mild form, characterized by flulike symptoms.
 - e. The urine antigen test is the most common laboratory assay used for the diagnosis of legionellosis.
 - f. Specimens from the lower respiratory tract, lung biopsy, bronchial wash, expectorated sputum, etc. are sometimes used for cultures for the diagnosis of the pneumonic form of the disease.
 - g. **Identifying characteristics**
 - 1) On a Gram stain, *Legionella* spp. appear as thin, poorly staining gram-negative bacilli. It is better to use 0.1% basic fuchsin as the counter stain instead of safranin.
 - 2) *Legionella* spp. **require L-cysteine for growth**. They will grow on **BCYE agar** but not on SBA. However, some species will grow on Brucella blood agar, a medium more nutritious than SBA. They can produce tiny colonies on chocolate agar.
 - 3) **They are asaccharolytic, and most biochemical tests are negative.**
 - 4) Most species will **autofluoresce** when exposed to ultraviolet light, including *L. pneumophila*.
 - 5) Other identifying tests: Direct fluorescent antibody test, urine antigen test, and nucleic acid probes
8. *Chromobacterium*
 - a. *Chromobacterium violaceum* is found in water and soil.

- b. Produces a purple or violet pigment on nutrient agar
 - c. Causes wound infections acquired from contaminated soil or water
9. ***Gardnerella vaginalis***
- a. *Gardnerella* are very small gram-variable coccobacilli. They differ from *Lactobacillus* spp., which are large gram-positive bacilli.
 - b. In low numbers, *G. vaginalis* is considered normal vaginal flora.
 - c. *G. vaginalis* is associated with **bacterial vaginosis (BV)**, UTIs, PID, and postpartum sepsis and may infect the newborn. *G. vaginalis* probably does not cause BV, but its presence is indicative of the condition.
 - d. Presence of **clue cells**, epithelial cells with numerous bacteria attached, is suggestive of BV.
 - e. Catalase negative
 - f. Amsel and Nugent scoring systems are used to diagnose BV. Cultures alone are too sensitive. Approximately 50–60% of women who do not meet the criteria for BV are positive for *G. vaginalis*.
10. ***Bartonella***
- a. Oxidase negative, gram-negative, curved bacilli
 - b. *Bartonella quintana*
 - 1) Agent of **trench fever**
 - 2) Also causes growth of neoplastic blood vessels in various parts of the body (bacillary angiomatosis) and other infections such as endocarditis
 - 3) Spread by human lice
 - c. *B. henselae*: Causes **cat-scratch disease** and also bacillary peliosis hepatitis and bacillary angiomatosis.
11. ***Cardiobacterium hominis***
- a. Found as normal flora in humans in the upper respiratory tract and possibly the gastrointestinal and genital tracts
 - b. Mainly associated with endocarditis
 - c. In Gram stains, *C. hominis* appears as short chains, pairs, or rosettes of irregularly staining bacilli with bulbous ends.
 - d. *C. hominis* requires CO₂ for initial isolation and can be recovered on SBA, although growth is enhanced in media containing yeast extract. It is oxidase positive, catalase negative, and weakly indole positive.
12. ***Streptobacillus moniliformis***
- a. Found as normal oral flora in rats and other rodents
 - b. Infections following animal bites results in a disease called **rat-bite fever**.
 - c. Ingestion of contaminated food or water results in **Haverhill fever**.
 - d. The bacteria are best isolated from blood, synovial fluid, and abscess material.
 - e. *S. moniliformis* is a nonmotile, facultative, gram-negative pleomorphic bacillus. It grows on media enriched with SBA (15% is optimal) incubated in a CO₂ incubator.

F. *Vibrio* and Similar Microorganisms

1. General characteristics

- a. Most are indole positive, and all are oxidase positive except *V. metschnikovii*.
- b. Some species cause GI disease.

2. *Vibrio*

- a. The genus contains about 12 species that are inhabitants of marine water.
- b. All species are **halophilic** (salt loving) except *V. cholerae* and *V. mimicus*.
- c. **Thiosulfate citrate bile salt sucrose agar (TCBS)** is a selective and differential (based on sucrose fermentation) medium that supports the growth of most species and is particularly useful for isolating *V. cholerae* and *V. parahaemolyticus*. *V. cholerae* is sucrose positive and will produce yellow colonies on TCBS agar, whereas *V. parahaemolyticus* is sucrose negative.
- d. Most laboratories use biochemical testing to presumptively identify species and then confirm with serology based on somatic O antigens.
- e. ***Vibrio cholerae***
 - 1) ***V. cholerae* O1** serological group causes cholera, characterized by severe watery diarrhea with flecks of mucus sometimes referred to as “**rice-water**” stool.
 - 2) **Serogroups non-O1** generally cause a mild choleralike illness. Serogroup O139 produces severe disease similar to *V. cholerae* O1.
 - 3) *V. cholerae* O1 is subdivided into three serotypes: Inaba, Ogawa, and Hikojima.
 - 4) *V. cholerae* O1 has two biotypes: classical and El Tor.
 - 5) Cholera infections are acquired by ingestion of undercooked seafood or contaminated drinking water. It is endemic to Southeast Asia, Africa, and South America.
 - 6) Important virulence mechanisms of *V. cholerae* include **cholera toxin** (cholera toxin, an enterotoxin), motility, pili, and mucinase.
 - 7) Symptoms seen in cholera are caused by an enterotoxin that alters ion transport of intestinal mucosa, resulting in a massive release of water.
 - 8) In addition to causing cholera, *V. cholerae* can also cause bacteremia, wound infections, and otitis media.
- f. ***Vibrio parahaemolyticus***
 - 1) Causes a mild to moderate choleralike diarrhea disease
 - 2) Acquired by eating raw shellfish
 - 3) Important cause of food poisoning in Asia, particularly in Japan and Taiwan
- g. ***Vibrio vulnificus***
 - 1) Highly virulent, causing septicemia after ingestion of undercooked seafood, notably raw oysters
 - 2) Causes a rapidly progressive wound infection after exposure to marine water

- h. *Vibrio alginolyticus*
 - 1) Very common in marine environment
 - 2) Suspected causes of otitis media and wound infections
- 3. *Aeromonas*
 - a. **Found in fresh and salt water**
 - b. Infects humans and fish
 - c. Causes cellulitis and diarrhea
 - d. Clinically important species include *A. hydrophila*, *A. caviae*, *A. veronii* biovar *sobria*, and *A. veronii* biovar *veronii*.
 - e. Generally cause a self-limiting infection not usually requiring treatment; however, wound infections may require antimicrobial therapy.
 - f. *A. hydrophila* is typically beta-hemolytic and oxidase, citrate, indole, VP, and ONPG positive.
- 4. *Plesiomonas shigelloides*
 - a. **Acquired by eating undercooked seafood**
 - b. *P. shigelloides* is primarily associated with a self-limiting gastroenteritis. Treatment is required only in immunosuppressed patients or other severe cases.
 - c. Based on DNA homology, *P. shigelloides* was recently moved to the family *Enterobacteriaceae*, despite being oxidase positive.
- 5. *Campylobacter*
 - a. *Campylobacter* spp. are a major cause of food poisoning, causing gastroenteritis, diarrhea, and septic arthritis.
 - b. Infection is acquired by eating undercooked contaminated poultry or other meat products.
 - c. *C. jejuni* causes most infections in this genus.
 - d. Part of many routine stool culture work-ups
 - e. **Identifying characteristics**
 - 1) Curved bacilli that may appear S-shaped or spiral on Gram stain
 - 2) **Most species are microaerophilic.**
 - 3) A number of **selective media** (e.g., charcoal cefoperzone deoxycholate agar and Campy-colistin vancomycin amphotericin B) are available for the isolation of *C. jejuni* and *C. coli* from stool specimens.
 - 4) ***C. jejuni* grows at 42°C but will grow slowly at 37°C.**
 - 5) They do not oxidize or ferment carbohydrates, and most human isolates are catalase and oxidase positive.
 - 6) On wet mount, they will show darting motility.
 - 7) Resistance to cephalothin and sensitivity to nalidixic acid has been used in the past for identification of *C. jejuni* and *C. coli*; however, because of variability in the sensitivity pattern, disk identification tests are no longer recommended.
 - 8) *C. fetus* is a rare cause of extraintestinal infections and does not grow at 42°C.

6. *Helicobacter pylori*

- a. *H. pylori* causes peptic and duodenal ulcers and has been linked to stomach cancer.
- b. Oxidase, rapid urease, and catalase positive
- c. The microorganism can be **isolated from gastric biopsy** on SBA, Brucella, and Skirrow's agars incubated microaerophilically. Selective media for enteric *Campylobacter* are **not** recommended.
- d. Other methods to determine *H. pylori* colonization include fecal antigen detection, urea breath test, and demonstration of urease activity in stomach biopsy material.

III. MYCOBACTERIA

A. General Characteristics

1. Cause **tuberculosis** (TB) and other diseases
2. Mycobacteria are slender, nonmotile, non-spore-forming, obligate aerobes.
3. There are about 50 species of *Mycobacterium*, 14 of which are pathogenic to humans.
4. It is necessary to decontaminate samples containing normal flora before culturing, and sputum must also be digested. Specimens from normally sterile sites (e.g., CSF, blood, etc.) do not require decontamination.
5. Mycobacteria resist Gram staining because of lipids in their cell wall that prevent penetration of crystal violet and safranin.
6. Mycobacteria are **acid-fast** and are referred to as **acid-fast bacilli** (AFB). The primary stain in the acid-fast stain is **carbol fuchsin**. The Ziehl-Neelsen stain requires heating during the staining step, whereas the Kinyoun's stain does not.
7. **Specimens**
 - a. **Lower respiratory tract:** Sputum and bronchial washings, usually 3 to 5 samples, are collected early in the morning on different days.
 - b. **Urine:** 3 to 5 different morning voids
 - c. Blood and bone marrow
 - d. Tissue and body fluids
8. Centers for Disease Control and Prevention acid-fast smear evaluation and reporting criteria, see Table 6-3 ■.
9. **Digestion and decontamination of sputum samples**
 - a. The mycobacteria are slightly more resistant to acids and alkalis than contaminating bacteria making up the normal flora. Therefore, mild treatments, such as 2% NaOH with *N*-acetyl-L-cysteine (NALC), are effective. Only specimens containing normal flora, such as sputum that contains bacteria from the oral cavity, need to be decontaminated.
 - b. NALC is a mucolytic agent that liquefies mucus in respiratory specimens, releasing mycobacteria.
 - c. NaOH increases the pH to a level that is antibacterial.

TABLE 6-3 REPORTING CRITERIA FOR AFB ON DIRECT SMEAR

Report	Fuchsin Stain (× 1000)
No AFB seen	0
Doubtful, repeat	1–2/300 fields
1+	1–9/100 fields
2+	1–9/10 fields
3+	1–9/field
4+	>9/field

10. Solid media

- Lowenstein-Jensen (LJ)** contains egg components for growth and malachite green to inhibit growth of normal flora.
- Lowenstein-Jensen-Gruft** is made selective by the addition of penicillin and naladixic acid. It is also supplemented with RNA.
- Middlebrook medium** is agar based and contains 2% glycerol to support the growth of *M. avium* complex (MAC). These media generally exhibit growth several days before egg-based media. Antimicrobials can be added to make the media selective for the mycobacteria.

11. Liquid media

- Middlebrook 7H9 broth** is often used to maintain stock cultures and prepare isolates for biochemical tests.
- Mycobacterium* growth index tube (MGIT®)** by Becton Dickinson (Franklin Lakes, NJ) contains a modified 7H9 broth. The large amount of oxygen in the broth quenches the fluorescence of a fluorochrome. As mycobacteria grow, they consume the oxygen, and the fluorochrome will fluoresce when exposed to ultraviolet light.
- BACTEC 460TB system** contains a radioactively labeled substrate that can be metabolized by mycobacteria. Growth of mycobacteria releases radioactive CO₂ that is detected by the instrument.

12. Runyon groups

- Except for members of the *M. tuberculosis* complex, the mycobacteria are placed into groups according to their growth rate and photoreactivity.
- Growth rate:** Rapid growers produce colonies on solid media within 1 week. Most common pathogens are slow growers, and weakly pathogenic species are rapid growers.

- c. **Photoreactivity**
 - 1) **Photochromogens** produce yellow to orange pigment only when exposed to light.
 - 2) **Scotochromogens** produce yellow to orange pigment in the light and in the dark.
 - 3) **Nonchromogens** (nonpigmented) do not produce pigment.
 - d. Members of Runyon **group 1** are slow growers and photochromogens. **Group 2** members are slow growers and scotochromogens. Mycobacteria that are slow growers and nonchromogens belong to **group 3**. **Group 4** contains the rapidly growing mycobacteria. Because of variation within the species, Runyon groups are no longer commonly used.
13. Biochemical tests for the identification of the mycobacteria
- a. **Catalase:** All mycobacteria typically produce catalase; however, there are different forms of catalase that can be differentiated in the laboratory.
 - 1) **Heat-sensitive catalase:** A suspension of a *Mycobacterium* sp. is heated at 68°C for 20 minutes. A 1:1 mixture of 30% hydrogen peroxide and 10% Tween 80 is added; after 5 minutes, the suspension is observed for bubbles. Members of the *M. tuberculosis* complex, including *M. tuberculosis* and *M. bovis*, are negative.
 - 2) **Semiquantitative catalase:** A 1 mL aliquot of a mixture of hydrogen peroxide and Tween 80 is added to a 2-week-old culture deep of mycobacteria. After 5 minutes, the height of the column of bubbles is measured. Members of the *M. tuberculosis* complex produce columns of bubbles <45 mm.
 - b. **Nitrate**
 - 1) In the test for nitrate reductase, NaNO_3 is added to a heavy suspension of mycobacteria. The suspension is incubated for 2 hours at 35°C, and then nitrate reagents (HCl, sulfanilamide, and *N*-naphthylethylenediamine dihydrochloride) are added. Formation of a pink color is a positive reaction.
 - 2) *M. tuberculosis* and *M. kansasii* are nitrate reductase positive, and most other *Mycobacterium* spp. are negative.
 - c. **Niacin**
 - 1) Niacin (nicotinic acid) is a precursor in the synthesis of NAD. Although all mycobacteria produce niacin, some species produce an excess amount that is excreted from the cell. Niacin accumulates in the medium and is detected by reacting with a cyanogen halide.
 - 2) *M. tuberculosis* is one of the few species positive for the accumulation of niacin.
 - d. **Growth on MacConkey agar**
 - 1) MacConkey agar without crystal violet is inoculated with a 7-day broth culture of the test organism. This is not the same formulation used for gram-negative bacilli.
 - 2) *M. fortuitum* and *M. chelonae* are the only mycobacteria able to grow on MacConkey agar in 5 days.

- e. Susceptibility to thiophene-2-carboxylic acid hydrazide (T2H): differentiates *M. bovis* (susceptible) from most other species (resistant).
- 14. **Nucleic acid assays:** Because of their slowly growing nature, rapid and specific nucleic acid assays are becoming more common in clinical laboratories for the identification of the mycobacteria.
 - a. **AccuProbe®** (Gen-Probe Inc.): This commercial assay has probes for *Mycobacterium tuberculosis* complex, *Mycobacterium avium* complex, *M. avium*, *M. intracellulare*, *M. kansasii*, and *M. goodii*. The assay is highly specific and takes less than 2 hours. It is approved for use on culture isolates.
 - b. **Strip assays:** Probes are immobilized onto a membrane strip and bind biotinylated-polymerase chain reaction products. These assays are also commercially available for culture isolates.
 - c. **Direct nucleic acid amplification:** Commercial amplification assays approved for use on respiratory specimens are available for detection of *M. tuberculosis*. The target is a region of the 16S rRNA gene.

B. Clinically Important *Mycobacterium*

1. *Mycobacterium tuberculosis*

- a. Causes **tuberculosis**, a chronic primarily lower respiratory tract disease
- b. Spread by person-to-person contact via infected droplets, dust, etc.
- c. Only a few bacteria are necessary to cause disease.
- d. **Primary tuberculosis**
 - 1) Infection begins in the middle or lower areas of the lungs.
 - 2) The bacteria can spread to the lymphatic system, CNS, and heart.
 - 3) Macrophages phagocytize the bacteria and form multinucleated cells, which are eventually surrounded by fibroid cells. Together the cells form granulomatous lesions called **tubercles**, which can be seen on chest X-rays. The lesions can calcify, at which point they are called “**ghon complexes**.” While the bacteria are contained within the granulomas, the patient is typically asymptomatic. This stage of the disease is called a **latent infection**.
 - 4) Primary TB may not lead to active TB in people with healthy immune systems.
- e. **Reactivation or secondary tuberculosis**
 - 1) Occurs in people who have had latent TB
 - 2) Reactivation, because of alteration in the cell-mediated immune response, can be triggered by poor nutrition, alcoholism, or hormonal factors associated with pregnancy and diabetes.
 - 3) Treatment requires long-term combination therapy, which can last up to 24 months. First-line drugs include isoniazid, rifampin, ethambutol, and pyrazinamide.

- f. **Multidrug-resistant *M. tuberculosis*** (MDR-TB), defined as simultaneous resistance to isoniazid and rifampin, was first discovered in 1991, and, if present, indicates a poor prognosis for recovery.
- g. **Extremely-drug-resistant *M. tuberculosis*** (XDR-TB) is defined as resistance to any fluoroquinolone, and at least one of three injectable second-line drugs (capreomycin, kanamycin, and amikacin), in addition to isoniazid and rifampicin.
- h. **Purified protein derivative (PPD):** Skin test that determines exposure to *M. tuberculosis*
 - 1) Antigen is composed of heat-killed, filtered, ammonium sulfate precipitated protein from *M. tuberculosis*.
 - 2) Injected intradermally and is examined at 48 hours for swelling (induration)
 - 3) A positive skin test indicates previous exposure to the bacteria but not necessarily active disease.
- i. Colonies on LJ medium appear nonpigmented (tan or **buff**), dry, heaped, and granular in 14–21 days at 37°C.
- j. Acid-fast stain often shows ropelike formations (cording) from broth culture.
- k. Niacin and nitrate positive, *p*-nitro- α -acetylamino- β -hydroxypropiophenone (**NAP**) susceptible
- 2. ***Mycobacterium leprae***
 - a. Agent of **Hansen disease** (leprosy)
 - b. **Cannot be grown on artificial media**
 - c. Diagnosis is based on characteristic skin lesions and visualizing AFB in lesions.
- 3. ***M. avium* complex**
 - a. *M. avium* and *M. intracellulare* are difficult to distinguish and are referred to as *M. avium* complex (MAC).
 - b. MAC may cause disseminated disease in immunosuppressed patients, such as patients with human immunodeficiency virus infection, producing lung infections, lymphadenitis, and intestinal infections.
 - c. MAC is a slowly growing nonchromogen.
- 4. ***M. kansasii***
 - a. ***M. kansasii*** causes pulmonary infections and is the most commonly isolated photochromogen in the U.S. It has been isolated from tap water around the world.
 - b. It causes a lung disease that resembles classic TB and rarely disseminates, except in patients with severe immunosuppression.
 - c. It is a slow grower and is nitrate and catalase positive.
- 5. ***Mycobacterium scrofulaceum*** causes cervical lymphadenitis and other types of infections predominantly in children. It is a slowly growing scotochromogen.

6. *Mycobacterium ulcerans*, *M. marinum*, and *M. haemophilium* have all been implicated in skin infections. Their predilection for surface areas and extremities of the body is related to their optimal growth temperature range of 30–32°C. *M. haemophilium* requires ferric ammonium citrate or hemin for growth and can be grown on chocolate agar.
7. *Mycobacterium bovis* is responsible for a zoonosis, producing pulmonary infections primarily in cattle and occasionally in humans. *M. bovis* is a nonchromogen like *M. tuberculosis*, but it is nitrate and niacin negative and sensitive to T2H.
8. *M. fortuitum*, *M. chelonae*, and *M. abscessus*, may cause abscesses, osteomyelitis, wound and lung infections; however, they are weakly virulent. These species are among the rapidly growing mycobacteria.
9. *Mycobacterium gordonae* is found in fresh water, including tap water, and is rarely pathogenic. It is a slowly growing scotochromogen often isolated as a contaminant.

IV. ANAEROBIC BACTERIA

A. General Characteristics

1. Anaerobic bacteria (i.e., obligate anaerobes) comprise most normal flora of the mucous membranes.
2. Suspect anaerobic bacteria in the following situations:
 - a. Foul odor (from gas production) and necrotic tissue
 - b. Anaerobic body sites, abscesses, and wounds
 - c. Surgical specimens
3. **Definitions**
 - a. **Obligate anaerobe:** Bacterium that cannot use oxygen for metabolism and oxygen is lethal to the microorganism
 - b. **Aerotolerant anaerobe:** Bacterium that cannot use oxygen but can grow in its presence
 - c. **Facultative anaerobe:** Bacterium that will use oxygen if it is present and can grow, albeit more slowly, without oxygen
 - d. **Obligate aerobe:** Bacterium that requires oxygen at concentrations found in room air, about 20%
 - e. **Microaerophile:** Bacterium that requires oxygen at concentrations of 5–10%
 - f. **Capnophile:** Bacterium that requires increased concentration of CO₂

B. Anaerobic Media

1. Media contain supplements that enhance anaerobic growth. Vitamin K is added to enhance the growth of *Prevotella* and *Porphyromonas*, and hemin is an added enrichment for *Bacteroides* and *Prevotella*.
2. **Centers for Disease Control and Prevention (CDC) anaerobic blood agar:** For general growth of all anaerobes

3. **Bacteroides bile esculin (BBE) agar:** Selective and differential medium used to culture and presumptively identify *Bacteroides fragilis*
4. **Kanamycin-vancomycin laked sheep blood (KVLB) agar:** Enriched selective medium for isolation of slowly growing anaerobes such as *Prevotella* and *Bacteroides*; laked blood enhances pigment formation
5. **Phenylethyl alcohol (PEA) agar:** Enriched and selective medium used to grow most anaerobes, including *Clostridium* and *Bacteroides*; inhibits the growth of facultative, anaerobic, gram-negative bacilli (e.g., *Enterobacteriaceae*)
6. **Columbia-colistin-naladixic agar with 5% sheep blood:** Inhibits gram-negative organisms and is used to grow most gram-positive anaerobes and facultative anaerobes
7. **Egg yolk agar** is used to detect proteolytic enzymes (lipase and lecithinase) produced by *Clostridium*. **Lecithinase** activity produces an opaque zone from the cleavage of lecithin releasing insoluble fats (diglyceride). **Lipase** cleaves lipids, releasing glycerol, which floats to the top of the medium producing a blue-green sheen (mother-of-pearl) on the agar surface.
8. **Broths with reducing agents**, such as thioglycolate and cooked (or chopped) meat, can be used to grow anaerobic bacteria. Sometimes **resazurin**, an oxidation-reduction indicator, is added. The indicator is pink in the presence of oxygen and colorless when reduced.
9. Solid media must be placed in **anaerobic conditions** in order for obligate anaerobes to grow.
 - a. Commonly used systems include anaerobic GasPak jars and bags and anaerobic hoods. In the presence of **palladium**, a catalyst, the following reaction occurs:

$$2\text{H}_2 + \text{O}_2 \rightarrow 2\text{H}_2\text{O}_2$$
 - b. An oxidation-reduction indicator (E_h) must be used to determine if anaerobic conditions have been met. **Methylene blue** is the most commonly used oxidation-reduction indicator. When anaerobic conditions are achieved, the methylene blue indicator will turn from blue (oxidized) to white, indicating reduction.
10. **Aerotolerance testing:** Before attempting to identify a possible anaerobic bacterium, it first must be demonstrated to be an obligate anaerobe. A colony is inoculated to an anaerobic blood agar plate, which is incubated anaerobically, and to a chocolate agar plate incubated under conditions of increased CO_2 . Isolates growing only on the plate incubated anaerobically are obligate anaerobes.

C. Gram Stain Morphology

1. *Bacteroides* and *Prevotella*: Pale, pleomorphic gram-negative coccobacilli with bipolar staining
2. *Fusobacterium*: Long, thin, filamentous gram-negative bacilli with tapered ends arranged end to end

3. *Actinomyces*: Branching gram-positive bacilli
4. *Clostridium*: Large gram-positive bacilli, spore location (terminal, central, or subterminal) is important in species identification

D. Biochemical Reactions

1. Important anaerobic biochemical tests include catalase, nitrate, urease, and indole.
2. Antimicrobial susceptibility disks can also be used to help identify anaerobes.

E. Anaerobic Gram-Negative Bacilli

1. ***Bacteroides fragilis* group**
 - a. ***B. fragilis* is a nonpigmented bacillus responsible for most anaerobic infections**, and many isolates are becoming more resistant to antimicrobial agents. A polysaccharide capsule is an important virulence mechanism.
 - b. **Major normal flora of the colon**
 - c. Causes infections by gaining entry into normally sterile body sites, especially after surgery, trauma, or disease
 - d. **Identifying characteristics**
 - 1) Nonmotile gram-negative bacilli with rounded ends and may be pleomorphic
 - 2) Nonhemolytic on anaerobic blood agar
 - 3) Biochemistry: **Growth in 20% bile**, catalase positive, lipase negative, bile-esculin positive, lecithinase negative, and gelatinase negative
 - 4) Produces brown to black colonies on BBE agar
 - 5) *B. fragilis* is resistant to penicillin, kanamycin, and vancomycin and susceptible to rifampin.
2. ***Prevotella melaninogenica***
 - a. **Pigmented saccharolytic gram-negative bacilli**
 - b. Normal flora of the oropharynx, nose, and GI and urogenital tracts
 - c. Causes head, neck, and lower respiratory tract infections
 - d. **Identifying characteristics**
 - 1) Young colonies appear tan and exhibit brick-red fluorescence under ultraviolet (UV) light. Older colonies are brown to black. It may take up to 3 weeks to see brown to black pigment.
 - 2) Biochemistry: Ferments glucose and many other carbohydrates and is inhibited by 20% bile
 - 3) Susceptible to rifampin and resistant to kanamycin
3. ***Porphyromonas***
 - a. **Asaccharolytic** or weak fermenters, pigmented colonies, gram-negative bacilli
 - b. Normal flora of the oropharynx, nose, and GI and urogenital tracts
 - c. Causes infections of the head, neck, oral cavity, and urogenital tract
 - d. **Identifying characteristics**
 - 1) Brick red fluorescence under UV light

2) *Porphyromonas* spp. will not grow on KVLB agar and are inhibited by bile, vancomycin, penicillin, and rifampin. However, they are resistant to kanamycin.

4. *Fusobacterium*

- a. Asaccharolytic or weak fermenters, nonpigmented colonies, gram-negative bacilli
- b. **Normal flora of the upper respiratory and GI tracts**
- c. *Fusobacterium* spp. cause pulmonary, blood, sinus, and dental infections in addition to brain abscesses. Many infections are associated with metastatic conditions.
- d. Two important species are *F. nucleatum* (causes serious pulmonary infections) and *F. necrophorum* (lung and liver abscesses, and arthritis). *F. nucleatum* is the more common isolate, but *F. necrophorum* causes more serious infections.
- e. **Identifying characteristics**
 - 1) Colony morphology: Opalescent with speckles
 - 2) Indole and lipase positive, nitrate and catalase negative
 - 3) Relatively biochemically inactive
 - 4) Inhibited by kanamycin and colistin, resistant to vancomycin

F. Anaerobic Gram-Positive Spore-Forming Bacilli

1. General characteristics of the *Clostridium*

- a. Some species are normal GI flora of humans and animals, and others are found in soil, water, and dust.
- b. Most species are anaerobic; a few are aerotolerant.
- c. Large gram-positive bacilli; some species appear gram-negative
- d. Catalase negative
- e. Most *Clostridium* species are motile; nonmotile species include *C. perfringens*, *C. ramosum*, and *C. inocuum*.
- f. Produce a variety of exotoxins

2. *Clostridium perfringens*

- a. *C. perfringens* is the most important pathogen in the genus. It causes **gas gangrene (myonecrosis)**, post-abortion sepsis, abdominal infections, and enterocolitis.
- b. Major cause of **food poisoning** (from meats and gravy), resulting in a mild to moderate diarrhea without vomiting
- c. Bacteria are acquired through puncture wound or by ingestion. *C. perfringens* is normal flora of the GI tract and can spread from this site following trauma.
- d. *C. perfringens* is also normal flora of the female genital tract and can cause post-abortion infections.
- e. Diabetics and patients with circulatory disorders are more prone to infection.

- f. *C. perfringens* secretes enzymes and exotoxins that cause severe tissue damage. This organism is divided into five types, A to E, based on the quantities and types of exotoxins produced.
- g. **Identifying characteristics**
 - 1) Produces a **double zone of beta-hemolysis on SBA** incubated anaerobically
 - 2) *C. perfringens* exhibits a positive (enhanced hemolysis) **reverse CAMP test**. In this assay, *Streptococcus agalactiae* (group B streptococci) is substituted for *Staphylococcus aureus* in the standard CAMP test.
 - 3) Positive for **lecithinase** and glucose, lactose, maltose, and fructose fermentation
 - 4) Spores are subterminal but difficult to induce.
 - 5) **Nonmotile**
 - 6) Nagler test: Antilecithinase antibody is swabbed onto half of an egg yolk agar plate. The isolate is inoculated onto both halves of the plate. *C. perfringens* produces lecithinase, which will produce an opaque zone on the half of the plate without the antibody. The antibody will neutralize lecithinase, preventing the opaque zone from forming. This test is not performed much today.
3. *Clostridium tetani*
 - a. Causes **tetanus**
 - b. *C. tetani* produces **tetanospasmin**, a neurotoxin that affects the anterior horn cells of the spinal cord, resulting in involuntary muscle contractions. Contractions begin with the neck and jaw (“**lock jaw**”) and progress to a backward arching of the back muscles.
 - c. Bacteria and spores gain entry into the host by puncture wounds contaminated with soil, or by wounds, which may include gunshots, burns, or animal bites. The bacteria produce little necrosis.
 - d. **Treatment and prevention:** Antitoxin and vaccine (DPT: diphtheria, pertussis, and tetanus trivalent vaccine) booster every 5 years
 - e. **Identifying characteristics**
 - 1) Gram-positive bacilli with round/terminal spores that resemble drumsticks
 - 2) Gelatinase, indole and motility positive, lecithinase and lipase negative
 - 3) Generally not cultured; diagnosis made by signs and symptoms and toxin detection
4. *Clostridium botulinum*
 - a. Causes **botulism**
 - b. **Botulism toxin** is a neurotoxin that binds to the synapse of nerve fibers, resulting in acute (flaccid) paralysis and death.
 - c. Botulism is usually acquired by ingestions of spoiled, home-canned foods in which the spores are not destroyed.
 - d. **Infant botulism** is the most common type of botulism. Bacteria are ingested and grow in the infant GI tract and can cause a rapidly fatal infection.

- e. **Identifying characteristics**
 - 1) Lipase, lecithinase, glucose, and motility positive
 - 2) Spores are oval/subterminal and resemble tennis rackets.
- 5. ***Clostridium difficile***
 - a. Causes antibiotic-associated pseudomembranous colitis and diarrhea
 - b. *C. difficile* is normal GI flora in a small percentage of the population, and as many as 30% of hospitalized patients may carry the bacteria.
 - c. High carriage rate in the intestines of patients who have received broad-spectrum antimicrobial agents that have eliminated the normal intestinal flora
 - d. **Produces enterotoxin A and/or cytotoxin B**
 - e. Infections can be diagnosed by detecting either toxin in the stool. The toxins can be detected using various immunologic methods, including enzyme immunoassay, and by examining cell monolayers for cytopathic effect after the addition of stool filtrates.
 - f. **Identifying characteristics**
 - 1) Because *C. difficile* can be normal flora, stool cultures can sometimes be too sensitive. Cultures for *C. difficile* should only be performed on watery or unformed stools. It is also important to test isolates for toxin production. Cycloserine-cefoxitin-fructose agar (CCFA) is used for isolating *C. difficile* from stool specimens. *C. difficile* is weakly fructose positive. Despite being fructose positive, the colonies are yellow. In reduced (i.e., anaerobic) conditions, the pH indicator turns yellow at a pH of about 5.3. The product will also fluoresce yellow-green.
 - 2) Lecithinase, lipase, and indol negative, and positive for motility and glucose and fructose fermentation
 - 3) Spores are oval and subterminal.
- 6. **Other *Clostridium* spp.** are infrequently associated with infections.
 - a. *C. septicum*, normal flora of the gastrointestinal tract, indicates colon cancer when isolated in blood cultures. This is a characteristic associated with the *Streptococcus bovis* group as well.
 - b. *C. septicum*, along with *C. perfringens*, is a member of the histotoxic group and is occasionally linked to gas gangrene.

G. Anaerobic Non-Spore-Forming Gram-Positive Bacilli

- 1. **Anaerobic *Actinomyces***
 - a. Normal flora of animal and human mucous membranes
 - b. ***A. israelii***, which causes abdomen and chest infections and pelvic actinomycosis in women with intrauterine devices, is the most common pathogen.
 - c. **Identifying characteristics**
 - 1) Exudate contains sulfur granules—dense clumps of bacteria.
 - 2) Gram-positive bacilli with a beaded appearance, often filamentous
 - 3) Colony morphology: Smooth to molar toothlike morphology

2. ***Propionibacterium***

- a. Species include *P. acnes* and *P. propionicus*.
- b. Often called anaerobic diphtheroids
- c. Normal flora of the skin, mouth, and GI tract
- d. Rarely pathogenic
- e. Catalase and indole positive

3. ***Mobiluncus***

- a. Associated with **BV**, PID, and abdominal infections
- b. Curved bacilli
- c. Motile, catalase and indole negative
- d. Inhibited by vancomycin

4. ***Lactobacillus***

- a. *Lactobacillus* is normal flora of the GI and female genital tracts. This organism helps to maintain an acidic environment in the vagina. If the population of lactobacilli decreases, the risk of BV increases.
- b. Rarely pathogenic
- c. Lactobacilli are generally aerotolerant anaerobes that will form small alpha-hemolytic colonies on SBA.
- d. Catalase negative and nonmotile bacilli

5. *Bifidobacterium*: Mostly nonpathogenic normal oral and intestinal flora

6. *Eubacterium*: Mostly nonpathogenic normal oral and intestinal flora

H. Anaerobic Gram-Positive and Gram-Negative Cocci

1. General characteristics of anaerobic cocci

- a. Normal flora of the intestines, female genital tract, oral cavity, and respiratory tract
- b. Associated with polymicrobial liver and brain abscesses and wound infections

2. **Anaerobic gram-positive cocci**

- a. ***Peptococcus***, the only species is *P. niger*
 - 1) Catalase positive
 - 2) Produces olive-green colonies that become black
- b. ***Peptostreptococcus***
 - 1) *P. anaerobius*, inhibited by **sodium polyanethol sulfonate (SPS)**
 - 2) *P. magnus* was renamed *Fingoldia magna*, and *P. asaccharolyticus* was renamed *Peptoniphilus asaccharolytica*.

3. **Anaerobic gram-negative cocci**

- a. ***Veillonella***
- b. **Identifying characteristics**
 - 1) Small, gram-negative cocci
 - 2) Reduces nitrate to nitrite, does not ferment any carbohydrates
 - 3) Inhibited by kanamycin and colistin but resistant to vancomycin

V. CHLAMYDIA, RICKETTSIA, AND MYCOPLASMA

A. *Chlamydia* and *Chlamydophila*

1. **Obligate intracellular parasites**
2. Cannot produce ATP; require ATP from host cell
3. Contain both DNA and RNA and are susceptible to antimicrobial agents
4. **Diagnosis**
 - a. **Cytological methods:** Detect chlamydia inclusions in epithelial cells
 - b. **Cell cultures** are required to grow the bacteria.
 - c. **Nucleic acid amplification tests (NAATs)** are the most common diagnostic method.
 - d. **Serology:** Antibody to lipopolysaccharide and outer membrane protein antigens
5. Three important species
 - a. *Chlamydia trachomatis*
 - 1) Causes lymphogranuloma venereum, trachoma, urethritis, conjunctivitis, and infant pneumonia
 - 2) **Trachoma is the leading cause of blindness in the world.**
 - 3) **No animal vectors;** it is spread by human-to-human contact.
 - 4) **Diagnosis:** Cell cultures, direct fluorescent antibody tests, enzyme immunoassays, NAATs, and serologic procedures
 - b. *Chlamydophila* (formerly *Chlamydia*) *pneumoniae*
 - 1) Mild respiratory tract infections producing flulike symptoms, may also cause Guillain-Barré syndrome
 - 2) No animal vectors; it is spread by human-to-human contact.
 - 3) **Diagnosis** is often made by using fluorescence-labeled *C. pneumoniae* antibodies.
 - c. *Chlamydiophila* (formerly *Chlamydia*) *psittaci*
 - 1) Causes **psittacosis** (ornithosis) or parrot fever, a disease of parrots, parakeets, cockatiels, and other birds such as turkeys and chickens
 - 2) Humans get infections by the inhalation of bird fecal dust; infections are uncommon in the U.S.
 - 3) **Incubation period 1–2 weeks:** Chills, fever, malaise, can progress to pneumonia, which can be fatal
 - 4) Occupational hazard to farmers, pet shop employees, and bird owners
 - 5) Diagnosis by serology

B. *Rickettsia* and Similar Genera

1. *Rickettsia* and *Ehrlichia* are **obligate intracellular parasites** requiring nucleotides and other metabolic building blocks from host cells.
2. **Infections are generally spread by insect vectors** (ticks, mites, and lice).

3. Diagnosis is often made by clinical symptoms, patient history, and serology. Immunohistology and polymerase chain reaction assays are also available.
4. Weil-Felix serologic test utilizes *Proteus* antigens. This assay is nonspecific and is not used much today.
5. The bacteria can be grown in embryonated eggs and tissue cells. However, cultures require a biosafety level 3 laboratory and, for safety concerns, cultures are not recommended.
6. **Clinically important species**
 - a. *R. rickettsii* causes Rocky Mountain spotted fever (RMSF) and is the most important species in the U.S. It is a member of the spotted fever group and is carried by ticks. RMSF is a very serious disease; death rates are approximately 25%.
 - b. *R. prowazekii* causes typhus, also called epidemic or louse-borne typhus; it is carried by human lice. Brill-Zinsser disease is a reactivation of the original infection.
 - c. *R. typhi* causes endemic or murine typhus. It is transmitted by fleas.
 - d. *Coxiella burnetii* causes Q fever. It is transmitted by inhalation, contact with fomites, and ingestion of contaminated milk.
 - e. *Ehrlichia chaffeensis* causes ehrlichiosis or human monocytic ehrlichiosis. It is transmitted by ticks and is endemic to the U.S.
 - f. *Anaplasma* (formerly *Ehrlichia*) *phagocytophilum* causes human granulocytic anaplasmosis.

C. *Mycoplasma* and *Ureaplasma*

1. Smallest free-living organisms, about the size of a large virus and beyond the resolution of light microscopes
2. They lack a cell wall, making them pleomorphic and resistant to all antibiotics that inhibit cell wall synthesis (e.g., beta-lactams).
3. They contain both RNA and DNA and can self-replicate.
4. Infections can be diagnosed by serology.
5. Many species of *Mycoplasma* and *Ureaplasma* grow on special laboratory media, including **SP4 and A8 agars and Shepard's 10 B broth**. *U. urealyticum* produces a strong alkaline pH because of the activity of **urease**. Some species will also grow on chocolate agar. Some species produce fried egg colony morphology.
6. **Clinically important species**
 - a. *Mycoplasma pneumoniae*
 - 1) Causes tracheobronchitis and community-acquired primary atypical (walking) pneumonia, resulting in a dry, nonproductive cough
 - 2) Spread by direct respiratory contact
 - 3) Mostly seen in teenagers and young adults; lacks a seasonal distribution
 - 4) *M. pneumoniae* produces hydrogen peroxide, which causes lysis of red blood cells *in vitro*.

- b. *M. hominis*
 - 1) Opportunistic pathogen linked to PID in sexually active adults
 - 2) May cause infant meningitis and postpartum fever
- c. *Ureaplasma urealyticum*
 - 1) Causes nongonococcal urethritis and may cause other genital tract infections
 - 2) Requires urea

VI. SPIROCHETES

A. Genera Causing Human Disease: *Treponema*, *Leptospira*, and *Borrelia*

1. Spirochetes are long, slender, helically curved bacilli that cannot usually be seen on Gram stain.
2. Special stains such as silver and Giemsa will stain spirochetes, silver for all spirochetes and Giemsa only for *Borrelia*.
3. Spirochetes can be observed by **darkfield** or **phase-contrast microscopy**.

B. *Treponema pallidum* subsp. *pallidum*

1. Causes **syphilis**
2. Transmitted by sexual contact, direct blood transmission, or transplacentally (congenital syphilis)
3. **Stages of syphilis**
 - a. **Primary:** Chancre at the site of inoculation
 - b. **Secondary:** Skin rash and lesions on oral mucosa
 - c. **Latent:** Absence of clinical symptoms
 - d. **Tertiary:** CNS disorders (neurosyphilis), aneurysms, and skin, liver, and bone disorders
4. *T. pallidum* **cannot be cultured in the laboratory**. The bacteria exhibit corkscrew motility seen by darkfield microscopy on material taken from lesions.
5. **Generally diagnosed by serology**
 - a. **Nontreponemal antigen tests** include the Venereal Disease Research Laboratory (VDRL) and rapid plasma reagin (RPR) tests, which detect antibodies to cardiolipin-lecithin-cholesterol and are nonspecific. These antibodies are sometimes referred to as **reagin**. Biologic false positives are caused by Lyme disease, various viruses, autoimmune disorders (e.g., systemic lupus erythematosus), and pregnancy.
 - b. **Treponemal antigen tests** include the fluorescent treponemal antibody absorption (FTA-ABS) test and the *Treponema pallidum* particulate antigen (TP-PA) test, which are specific and confirmatory.
6. Other clinically important species include *T. pallidum* subsp. *pertenue* (yaws), *T. pallidum* subsp. *endemicum* (endemic syphilis), and *T. carateum* (pinta).

C. *Borrelia*

1. *Borrelia recurrentis*

- a. *B. recurrentis* causes **epidemic relapsing fever**, which is characterized by recurrent high fever, chills, muscle pain, and headache. Other *Borrelia* spp. cause endemic relapsing fever transmitted by arthropods such as ticks.
- b. Humans are the only known reservoir for this species; bacteria are transmitted by body lice.
- c. *Borrelia* spp. are difficult to culture, and serological tests are insensitive.
- d. **Diagnosis** is based on observing bacteria in the peripheral blood-stream via the Giemsa or silver stains, or by darkfield microscopy. Due to low bacterial numbers, it can be difficult to diagnose infections by staining.

2. *Borrelia burgdorferi*

- a. Causes **Lyme disease**, also known as Lyme borreliosis
- b. ***B. burgdorferi* is the most common tickborne disease in the U.S.** It is transmitted by the deer tick (*Ixodes dammini*).
- c. **Stages of Lyme disease**
 - 1) **Early localized (stage I):** A rash at the bite site (**erythema migrans**) produces a characteristic “bull’s eye” pattern in many patients.
 - 2) **Early disseminated (stage II):** Bacteria enter the blood stream (producing flulike symptoms) and then can go to the bones (arthritis), CNS (meningitis, paralysis), or heart (palpitations, carditis). Patients present with fatigue, malaise, arthralgia, myalgia, and headaches.
 - 3) **Late stage (stage III):** This stage is characterized by chronic arthritis and acrodermatitis that can continue for years.
- d. **Diagnosis**
 - 1) Serologic tests are sensitive in diagnosing Lyme disease. **Western immunoblotting** is considered the most accurate method for antibody detection.
 - 2) Difficult to culture and too few bacteria to detect by direct microscopy

D. *Leptospira*

1. *L. interrogans* causes leptospirosis (Weil’s disease).
2. **Zoonosis of rodents, dogs, and cattle**
3. **Humans acquire the infection by contact with contaminated animal urine.**
4. The infection can produce fever, kidney, liver, and CNS involvement.
5. **Diagnosis of leptospirosis**
 - a. Direct examination via darkfield microscopy, or with silver stain

- b. Microorganisms can be recovered in cultures. Blood is the most sensitive specimen during early infections. Urine should be cultured after the second week. Media include Ellinghausen-McCullough-Johnson-Harris and Fletcher's.
- c. Most cases are diagnosed by serology, although methods vary in sensitivity.

VII. ANTIMICROBIAL AGENTS AND ANTIMICROBIAL SUSCEPTIBILITY TESTING

A. Definitions

1. An **antibiotic** is a molecule produced by microorganisms that inhibits the growth of other microorganisms. Antibiotics can also be synthetic.
2. **Cidal**: Kills microorganisms (e.g., bactericidal compound kills bacteria)
3. **Static**: Inhibits the growth of microorganisms (e.g., bacteristatic compound inhibits bacterial growth)
4. **Synergy**: When two or more antimicrobials are used and the combined effect is greater than what would be expected for the simple additive effect of the agents

B. Spectrum of Action

1. Narrow-spectrum antimicrobial agent: Limited range of action
2. Broad-spectrum antimicrobial agent: Active against a wide range of bacteria

C. Classes of Antimicrobial Agents and Their Mode of Action

1. **Beta-lactam antibiotics** inhibit cell wall synthesis (e.g., penicillins, cephalosporins, monobactams, and carbapenems). The class cephalosporin contains a large number of agents categorized as **narrow spectrum** (first generation), **expanded spectrum** (second generation), **broad spectrum** (third generation), and **extended spectrum** (fourth generation).
2. **Beta-lactamase inhibitors**: Bacteria can exhibit resistance to the beta-lactam antibiotics by producing an enzyme (**beta-lactamase**) that cleaves the beta-lactam ring, inactivating the antibiotic. Beta-lactamase inhibitors can be given with a beta-lactam antibiotic to provide effective treatment. Clavulanic acid, sulbactam, and tazobactam are examples of beta-lactamase inhibitors.
3. **Aminoglycosides** inhibit protein synthesis at the 30S ribosomal subunit and are active against gram-negative and gram-positive bacteria (e.g., gentamicin, tobramycin and netilmicin). Tobramycin is bactericidal whereas the others are bacteristatic. They have no activity against obligate anaerobes. Because of potential toxicity, dosage should be monitored using peak and trough values in peripheral blood.
4. **Tetracyclines** inhibit protein synthesis at the 30S ribosomal subunit (e.g., doxycycline and minocycline). They are active against gram-positive and gram-negative bacteria and *Mycoplasma* and *Chlamydia*. Increased resistance has limited their use.

5. **Chloramphenicol** inhibits protein synthesis by binding to the 50S ribosomal subunit. It is broad spectrum and used to treat serious gram-negative infections such as meningitis. Risk of bone marrow toxicity, aplastic anemia (bone marrow suppression), limits use to serious infections.
6. **Macrolides** inhibit protein synthesis (e.g. erythromycin and clarithromycin).
7. **Sulfonamides** inhibit folic acid synthesis by forming nonfunctional analogs of folic acid.
8. **Glycopeptides** inhibit cell wall formation by inhibiting peptidoglycan synthesis; vancomycin is the only glycopeptide approved for use in the U.S. Vancomycin-resistant enterococci, vancomycin-intermediate *S. aureus*, and vancomycin-resistant *S. aureus* have been isolated.
9. **Quinolones** inhibit DNA activity by inactivating DNA gyrase. Newer agents are known as fluoroquinolones (e.g., ciprofloxacin and levofloxacin).
10. **Polymyxins** disrupt plasma membranes; they are used to treat infections caused by gram-negative bacteria (e.g., polymixin B and polymixin E).
11. **Nitrofurantoin** inhibits bacterial enzymes; nitrofurantoin is used to treat UTIs.

D. Antimicrobial Susceptibility Testing

1. Dilution tests

- a. In these assays, bacteria are exposed to different concentrations of antimicrobial agents. The smallest concentration that inhibits growth of the bacteria is recorded; this value is the **minimal inhibitory concentration** (MIC).
- b. **Broth dilutions:** Dilutions of the antimicrobial agents are prepared in broth. The assays are generally performed in microtiter plates.
- c. **Agar dilutions:** Dilutions of the antimicrobial agents are prepared in agar. Bacteria are inoculated onto the agar plates.
- d. **The minimum bactericidal concentration** (MBC) of an antimicrobial agent is defined as the lowest concentration of an antimicrobial agent that kills at least 99.9% of the bacteria in the original inoculum. This can be determined by first performing a broth dilution test and then subculturing the tubes without visible growth to media without antimicrobial agents. The sample taken from the tube with the lowest concentration of antimicrobial agent showing no growth is representative of the MBC.

2. Disk diffusion

- a. Also referred to as **Kirby-Bauer sensitivity test**
- b. **Standardization**
 - 1) **Mueller-Hinton agar (MHA)**, 4 mm thick in Petri dish at a pH 7.2–7.4, is required. In the case of fastidious microorganisms (e.g., *Streptococcus pneumoniae*), MHA with 5% sheep red blood cells is used. For *Haemophilus influenzae*, *Haemophilus* test medium (HTM) is used. HTM is Mueller-Hinton base supplemented with hematin, NAD, and yeast extract.

- 2) **Bacterial inoculum**, 10^8 colony forming units/mL, which is equal to a McFarland #0.5 turbidity standard
- 3) MHA plates are incubated for 18 hours at 35°C in ambient air. Both HTM and MHA with sheep red blood cells are incubated in 5–7% CO_2 for 18–20 hours.
- c. After incubation, the **diameters of the zones of inhibition** are measured. The zone sizes are compared to standard interpretation charts, and the results are reported as **sensitive (S), intermediate (I), or resistant (R)**.
- d. **Quality control organisms** vary depending on the susceptibility test used.
- e. **Detection of MRSA**
 - 1) Methicillin is no longer available in the U.S., so when referring to MRSA, it is actually oxacillin or nafcillin resistance that is being discussed.
 - 2) Because populations of MRSA are often heteroresistant (some cells sensitive and much fewer resistant), testing procedures should be modified to be sensitive for the detection of MRSA.
 - 3) Cefoxitin is a more powerful inducer of oxacillin resistance and can be used in disk diffusion assays. The procedure is the same as for routine disk diffusion except that interpretive criteria are changed: for *S. aureus*, zones of ≤ 19 mm are reported as oxacillin resistant and results ≥ 20 mm are reported as sensitive.
 - 4) In broth dilution tests with oxacillin, *S. aureus* isolates with MICs < 2 $\mu\text{g/mL}$ are considered sensitive and results > 4 $\mu\text{g/mL}$ are resistant.
3. **Gradient diffusion**
 - a. **Etest®** (AB Biodisk) provides quantitative antimicrobial susceptibility testing results.
 - b. Procedure
 - 1) A bacterial suspension equal to a McFarland #0.5 turbidity standard is prepared.
 - 2) The bacteria are lawned onto a Mueller-Hinton agar plate and the Etest strips are placed on top of the agar. Each strip contains a different antimicrobial agent.
 - 3) After incubation, the bacteria produce an elliptical zone of inhibition around the strip. The MIC is read from a scale on the strip where the zone of inhibition crosses the strip.
4. **Miscellaneous assays**
 - a. **Beta-lactamase** is an enzyme that confers resistance to penicillin and some of the semisynthetic penicillins (e.g., ampicillin). Several methods are available for detecting the presence of beta-lactamase. Some *Enterobacteriaceae* can produce an **extended-spectrum beta-lactamase (ESBL)**. These enzymes inactivate the extended spectrum cephalosporins such as ceftriaxone and cefotaxime.

- b. The **D-zone test** is used to detect the presence of inducible clindamycin resistance by erythromycin. Even though clindamycin and erythromycin are in different classes, the mechanisms of resistance are similar. A plate is inoculated as for a disk diffusion assay. A 15- μ g erythromycin disk is placed 15 to 20 mm from a 2- μ g clindamycin disk. After incubation, the plate is examined for a flattening of the zone of inhibition around the clindamycin disk, resembling the letter D, indicating the presence of inducible resistance to clindamycin.

VIII. PROCEDURES AND BIOCHEMICAL IDENTIFICATION OF BACTERIA

A. Plating Procedures

1. General information

- a. The clinical specimen and the suspected pathogens will determine the selection of the primary plating media.
- b. The media used will vary among different laboratories because of local pathogens and personal preference of the laboratorians.

2. Clinical specimens

a. Blood

- 1) Blood is normally sterile.
- 2) Definitions and characteristics
 - a) **Bacteremia:** Bacteria in the blood
 - b) **Septicemia:** Bacteria increasing in numbers in the blood causing harm to the patient
 - c) When drawing blood cultures, avoid skin contamination and collect sample, if possible, before antimicrobial therapy.
 - d) Bacteria are in highest numbers in the blood just before fever spikes. It is important to collect several specimens at different times for greatest potential of bacterial yield (sensitivity). **The volume of blood collected probably has the greatest effect on isolation of bacteria.**

3) Cultures

- a) Blood culture systems utilize bottles containing liquid media.
- b) Generally, two bottles are inoculated: one for aerobes and one for obligate anaerobes. However, because of the reported decrease in the incidence of anaerobic bacteremias, a number of hospitals have stopped using anaerobic bottles.
- c) Most aerobic bottles contain 5–10% CO₂.
- d) Blood culture bottles often contain sodium polyanethol sulfonate (**SPS**), an anticoagulant that also inhibits complement and inactivates neutrophils. SPS has been shown to inhibit the growth of some bacteria.

b. Cerebrospinal fluid

- 1) CSF surrounds the brain and spinal cord and carries nutrients and waste; it is normally sterile.

- 2) Meningitis is an inflammation of the meninges.
- 3) Encephalitis is an inflammation of the brain.
- 4) The most common isolates found in CSF are *Neisseria meningitidis*, *Streptococcus pneumoniae*, *Streptococcus agalactiae*, *E. coli*, *Staphylococcus aureus*, and *Listeria monocytogenes*.
- 5) Diagnoses are made by a direct Gram stain and culturing on SBA, MAC, and chocolate agars.

c. **Throat**

- 1) *S. pyogenes* (group A *Streptococcus*) is the most important pathogen isolated in throat cultures; group B streptococci, group C streptococci, group G streptococci, and *Arcanobacterium* spp. are also clinically significant. Screening for other pathogens may occur upon request.
- 2) Alpha-hemolytic streptococci viridans group, *Neisseria* spp., *Corynebacterium* spp., and coagulase negative staphylococci make up the majority of the normal oral flora.
- 3) Culture on SBA and other media as needed by special request

d. **Sputum**

- 1) Used to diagnose lower respiratory tract infections (e.g., pneumonia)
- 2) The lower respiratory tract is normally sterile. However, sputum from the lungs acquires normal flora passing through the oral cavity.
- 3) A **direct Gram stain** is performed to determine the quality of the specimen. Acceptable specimens are cultured on SBA, MAC, and chocolate agars.
- 4) Several methods are used to determine specimen acceptability. Typically, squamous epithelial cells are an indication of contamination with oral flora, whereas polymorphonuclear cells (PMNs) indicate a quality specimen. A general rule for an acceptable specimen might be <10 squamous epithelial cells and >25 PMNs/low power field. This does not pertain to neutropenic or atypical pneumonia samples, which often have nonpurulent sputum.
- 5) Common significant sputum isolates
 - a) *Streptococcus pneumoniae* is an important cause of community-acquired pneumonia, and it is the most common cause of pneumonia in geriatric patients.
 - b) *Klebsiella pneumoniae* is associated with nosocomial pneumonia and pneumonia in alcoholics.
 - c) *Staphylococcus aureus* causes community-acquired and nosocomial pneumonia, usually secondary to another infection or predisposing factor.
 - d) *Pseudomonas aeruginosa* causes nosocomial and severe pneumonia in patients with CF.
 - e) *Haemophilus influenzae* causes infection in infants, children, and the immunosuppressed. The incidence of infections has decreased dramatically since routine use of the Hib vaccine.

- f) *Legionella pneumophila* primarily infects middle-aged males. *Legionella* spp. will not grow on routinely used media (i.e., SBA, chocolate, and MAC).
- g) *Mycoplasma pneumoniae* causes primary atypical pneumonia, which is mostly seen in young adults. *Mycoplasma* will not grow on routinely used media.

e. **Urine**

- 1) Urine is normally sterile.
- 2) **Bacteriuria** is bacteria in the urine, but it may not indicate a UTI.
- 3) Calibrated loops are used to determine colony counts on media.
- 4) Urine specimens are generally plated onto SBA and MAC or EMB.
- 5) Common significant urine isolates include *E. coli*, *Klebsiella* spp., *Enterobacter* spp., *Proteus* spp., *Staphylococcus aureus*, *Staphylococcus saprophyticus*, *Enterococcus* spp., *Pseudomonas aeruginosa*, and yeast.

f. **Stool**

- 1) Feces contain many species of anaerobic and facultative anaerobic normal flora.
- 2) Bacteria causing gastroenteritis include *Shigella* spp., *Salmonella*, *Campylobacter jejuni*, *E. coli* (e.g., O157:H7), *Yersinia enterocolitica*, *Clostridium difficile* (must test for cytotoxin), and *Vibrio* spp.
- 3) Plating protocols vary widely but in general include selective and differential media for the isolation and screening of specific pathogens.

g. **Genital tract**

- 1) Laboratorians commonly look for *Neisseria gonorrhoeae* and *Chlamydia trachomatis*.
- 2) The cervix is typically a sterile site. The vagina contains normal flora that changes with age. *Lactobacillus* spp. are the predominant flora during childbearing years. Earlier and late in life, staphylococci and corynebacteria predominate.
- 3) Types of genital tract infections
 - a) **Cervicitis** and **urethritis** usually caused by *N. gonorrhoeae* and *C. trachomatis*
 - b) **BV**, or nonspecific vaginitis, is due to overgrowth of some species of normal vaginal flora, most likely *Mobiluncus*. There is a corresponding decrease in lactobacilli. *Gardnerella vaginalis* is considered normal vaginal flora and may only be an indicator of BV.
 - c) **PID** is a complication of infection caused by *N. gonorrhoeae* or *C. trachomatis* involving the endometrium or fallopian tubes.
 - d) **Prostatitis** is usually caused by enterics.
- 4) Plating protocols for *N. gonorrhoeae* include using specific **selective media** (e.g., modified Thayer-Martin).

- 5) Molecular techniques are commonly used for detecting both *N. gonorrhoeae* and *C. trachomatis*.
- h. **Wounds/abscesses**
 - 1) Superficial skin infections: *Staphylococcus aureus* and *Streptococcus pyogenes*
 - 2) Folliculitis (hair follicle infection): *S. aureus* and *Pseudomonas aeruginosa*
 - 3) Boils, bedsores, etc.: *S. aureus*
 - 4) Impetigo: *S. pyogenes* and *S. aureus*
 - 5) Erysipelis: *S. pyogenes* and less commonly *Erysipelothrix rhusiopathiae*
 - 6) Deep and surgical wounds and abscesses: Anaerobes from normal body sites

B. Biochemical Identification of Bacteria

1. Catalase test

- a. Catalase is an enzyme that produces water and oxygen from hydrogen peroxide (H_2O_2). Several drops of H_2O_2 are added to a bacterial smear on a microscope slide.
- b. If catalase is present, water and oxygen (bubbles) will form. Staphylococci are positive and streptococci are negative.

2. Coagulase test

- a. **Clumping factor** (slide coagulase): Formerly slide coagulase tests used rabbit plasma. Clumping indicates a positive reaction and identification of *S. aureus*. However, *S. lugdunensis* and *S. schleiferi* can also produce positive results. Newer tests are based on latex agglutination and detect protein A in the cell wall and have higher sensitivity and specificity for *S. aureus*.
- b. The **tube coagulase test** uses rabbit plasma like the slide method, but it is incubated at 37°C for up to 24 hours. The human and animal pathogen *S. aureus* is positive. *S. intermedius* and *S. hyicus* are animal pathogens that are also positive. Tests must be checked at 4 hours for clot formation. Some strains produce staphylokinase, which can dissolve the clot, producing a false negative result.

3. PYR test

- a. This test detects the enzyme L-pyrrolidonyl arylamidase. A colony is placed on filter paper with the substrate pyrrolidonyl- α -naphthylamide (PYR).
- b. A red color after the addition of *p*-dimethylaminocinnamaldehyde (DMACA) is a positive PYR test. *Streptococcus pyogenes* and *Enterococcus* spp. are typically positive. The PYR test can also be used to differentiate *S. aureus* (negative) from *S. lugdunensis* and *S. schleiferi*, both of which are positive.

4. **Bile solubility test**

- a. Colonies of *Streptococcus pneumoniae* are soluble in sodium deoxycholate (bile).
- b. In the presence of the bile at 37°C, the colonies autolyse within 30 minutes, and disappear from the agar surface.

5. **Hippurate hydrolysis test**

- a. The hippurate hydrolysis test detects the bacterial enzyme hippuricase, which hydrolyzes hippurate to glycine and benzoic acid.
- b. A positive hippurate will give a purple color after the addition of ninhydrin.
- c. Group B streptococci are hippurate positive, whereas most other beta-hemolytic streptococci are negative. In addition, the test can be used to differentiate *Campylobacter jejuni* (positive) from most other *Campylobacter* spp.

6. **Oxidase test**

- a. The oxidase test detects cytochrome oxidase that is used in the electron transport system. Several drops of oxidase reagent (tetramethyl-*p*-phenylenediamine dihydrochloride) are placed on filter paper containing bacterial colonies or directly on plate colonies.
- b. Colonies should be taken from nonselective, nondifferential media. Media with a high concentration of glucose can inhibit oxidase activity.
- c. A positive oxidase test is indicated by a purple color within 10 to 15 seconds.

7. **Indole test**

- a. The indole test detects the bacterial enzyme **tryptophanase**. Tryptophan is broken down by tryptophanase into pyruvic acid, ammonia, and indole.
- b. Indole is detected by an aldehyde indicator (Ehrlich's reagent), yielding a red color, or Kovac's reagent, yielding a bright pink color. The Ehrlich method is more sensitive but requires an extraction step with xylene.
- c. A **spot indole test**, using DMACA, has been shown to be more sensitive in detecting indole activity. The presence of a blue to blue-green color is positive. Colonies from media containing dyes (e.g., eosin methylene blue and MAC) should not be tested because of the risk of a false positive result.

8. **Urease test**

- a. Urease breaks down urea to form ammonia (NH₃). Organisms are inoculated onto a urea agar slant and incubated at 37°C for 18–24 hours.
- b. A positive urease test is indicated by a bright pink color.

9. **Triple sugar iron agar (TSI)**

- a. TSI will show the pattern of glucose, lactose, and sucrose fermentation, in addition to H₂S and gas production.
- b. **Phenol red** is the pH indicator. The color of uninoculated medium is reddish-orange, yellow is acid, and red is alkaline.
- c. **Alkaline slant/alkaline deep (K/K)**: Nonfermenter, not *Enterobacteriaceae*

- d. **Alkaline slant/acid deep (K/A):** Nonlactose and nonsucrose fermenter, glucose fermenter
- e. **Acid slant/acid deep (A/A):** Lactose and/or sucrose fermenter, and glucose fermenter
- f. **Black deep, production of H_2S gas:** Test systems detect enzymes that produce hydrogen sulfide (H_2S) from sulfur-containing molecules in the medium. H_2S reacts with iron salts in the medium to form a black precipitate composed of ferrous sulfide.
- g. **Lead acetate** is a more sensitive method to detect H_2S gas. Lead acetate is added to filter paper strips. After the slant is inoculated, one end of the strip is held in place by the cap. If H_2S gas is produced, it reacts with the lead acetate, forming a black color (lead sulfide).
10. **IMViC** (indole, methyl red, Voges-Proskauer, and citrate)
 - a. **Indole:** Bacteria positive for indole produce tryptophanase, which breaks down tryptophan to pyruvic acid, ammonia, and indole. A pink color is a positive reaction, see above.
 - b. **Methyl red (MR):** MR is a pH indicator; it is yellow at an acid pH, indicating glucose fermentation. Red is negative.
 - c. **Voges-Proskauer (VP):** A positive VP reaction detects the metabolism of glucose to acetyl-methyl-carbinol (acetoin). Alpha-naphthol followed by 40% KOH is used to detect acetoin. Red is positive, and yellow is negative. Bacteria are usually MR or VP positive.
 - d. **Citrate:** This test determines if citrate is used as a sole carbon source. Blue is positive and green is negative.
11. **ONPG (*o*-nitrophenyl- β -D-galactopyranoside)**
 - a. This test detects the presence of β -galactosidase, an enzyme that cleaves ONPG and lactose. This test is useful in detecting delayed (late) lactose fermenters that lack, or are deficient in, beta-galactoside permease.
 - b. Yellow is a positive reaction, indicating the ability to ferment lactose.
12. **Amino acid degradation test**
 - a. A positive test detects bacterial enzymes that break down various amino acids. The color of positive and negative reactions depends on the pH indicator used.
 - b. **Deaminase reaction:** Detects the ability of an organism to remove the amino group from specific amino acids
 - c. **Decarboxylation reaction:** Detects the ability of bacteria to remove the carboxyl group from a specific amino acid
 - d. **Examples** include tryptophan (tryptophan deaminase), lysine (lysine decarboxylase), and ornithine (ornithine dihydrolase).
13. **Carbohydrate fermentation test**
 - a. A positive test detects the ability of bacteria to produce organic acids by the fermentation of various carbohydrates.

- b. Positive and negative reactions depend on the pH indicators used. Tubed media are inoculated and overlaid with sterile mineral oil to produce an anaerobic environment.

14. Nitrate reduction test

- a. A positive test determines the ability of an organism to reduce nitrate (NO_3) to nitrite (NO_2) and nitrogen gas (N_2).
- b. After the addition of the reagents (*N,N*-dimethyl- α -naphthylamine and sulfanilic acid), a pink color is positive for reduction of NO_3 to NO_2 . A colorless reaction requires the addition of zinc dust to confirm a negative result. Development of a pink color after adding zinc indicates a true negative. Remaining colorless after adding the addition of zinc indicates that NO_3 was completely reduced to N_2 , a positive result for nitrate reduction.

C. Multitest Systems

1. Most biochemical testing is performed using multitest methodologies. Semiautomated systems, such as the Vitek and Microscan, have identification and minimal inhibitory concentration combination plates.
2. Multitest systems
 - a. API (Analytical Profile Index; bioMérieux Clinical Diagnostics)
 - b. Enterotube II (Becton, Dickinson and Company)
 - c. Micro-ID (Remel)
 - d. Vitek (bioMérieux Clinical Diagnostics)
 - e. Microscan (Siemens Healthcare Diagnostics, formerly Dade Behring)

D. MIDI, Inc. Identification Systems

1. The Sherlock Microbial Identification System (MIDI, Inc.) identifies the fatty acid composition of the bacterial cell wall as determined by gas chromatography.
2. The fatty acids in mycobacteria have a larger molecular weight and are identified via high-performance liquid chromatography in the Sherlock Mycobacteria Identification System.



review

questions

INSTRUCTIONS

Each of the questions or incomplete statements that follows is comprised of four suggested responses. Select the *best* answer or completion statement in each case.

Aerobic Gram-Positive Bacteria

1. A test for the hydrolysis of esculin in the presence of bile is especially useful in identifying species of the genus
 - A. *Abiotrophia*
 - B. *Corynebacterium*
 - C. *Enterococcus*
 - D. *Staphylococcus*
2. The organism associated with a disease characterized by the presence of a pseudomembrane in the throat and the production of an exotoxin that is absorbed into the bloodstream with a lethal effect is
 - A. *Arcanobacterium haemolyticum*
 - B. *Staphylococcus aureus*
 - C. *Streptococcus pyogenes*
 - D. *Corynebacterium diphtheriae*
3. Enterotoxin produced by *Staphylococcus aureus* is responsible for causing
 - A. Carbuncles
 - B. Enterocolitis
 - C. Impetigo
 - D. Scalded skin syndrome
4. *Abiotrophia*, formerly known as nutritionally variant streptococci, will not grow on routine blood or chocolate agars because they are deficient in
 - A. Hemin
 - B. Pyridoxal
 - C. Vitamin B₁₂
 - D. Thiophene-2-carboxylic hydrazide
5. Exfoliatin produced by *Staphylococcus aureus* is responsible for causing
 - A. Enterocolitis
 - B. Toxic shock syndrome
 - C. Scalded skin syndrome
 - D. Staphylococcal pneumonia
6. *Streptococcus pyogenes* can be presumptively identified using a(an)
 - A. PYR disk
 - B. ONPG disk
 - C. SPS disk
 - D. Optochin disk

7. A gram-positive coccus that is catalase positive, nonmotile, lysostaphin resistant, and modified oxidase positive is best identified as a member of the genus
 - A. *Micrococcus*
 - B. *Lactococcus*
 - C. *Pediococcus*
 - D. *Staphylococcus*
8. *Nocardia asteroides* infections in humans characteristically produce
 - A. Carbuncles
 - B. Draining cutaneous sinuses
 - C. Septic shock
 - D. Serous effusions
9. *Erysipelothrix* infections in humans characteristically produce
 - A. Pathology at the point of entrance of the organism
 - B. Central nervous system pathology
 - C. Pathology in the lower respiratory tract
 - D. The formation of abscesses in visceral organs
10. In the CAMP test, a single streak of a beta-hemolytic *Streptococcus* is placed perpendicular to a streak of beta-lysin-producing *Staphylococcus aureus*. After incubation, a zone of increased lysis in the shape of an arrowhead is noted; this indicates the presumptive identification of
 - A. *S. agalactiae*
 - B. *S. bovis*
 - C. *S. equinus*
 - D. *S. pyogenes*
11. *Staphylococcus saprophyticus*, a recognized pathogen, is a cause of
 - A. Furuncles
 - B. Impetigo
 - C. Otitis media
 - D. Urinary tract infections
12. Color Plate 26■ shows the Gram stain of a blood culture on a 23-year-old pregnant woman who presented with fever and flulike symptoms in her ninth month. The isolate on blood agar produced small, translucent beta-hemolytic colonies. Which of the following is the most likely etiologic agent in this case?
 - A. *Listeria monocytogenes*
 - B. *Propionibacterium acnes*
 - C. *Streptococcus agalactiae*
 - D. *Streptococcus pyogenes*
13. The etiologic agent most commonly associated with septicemia and meningitis of newborns is
 - A. *Streptococcus agalactiae*
 - B. *Streptococcus bovis* group
 - C. *Streptococcus pneumoniae*
 - D. *Streptococcus pyogenes*
14. Which of the following is the most commonly isolated species of *Bacillus* in opportunistic infections such as bacteremia, post-traumatic infections of the eye, and endocarditis?
 - A. *B. circulans*
 - B. *B. cereus*
 - C. *B. licheniformis*
 - D. *B. subtilis*
15. Loeffler's serum medium is recommended for the cultivation of
 - A. *Abiotrophia* sp.
 - B. *Corynebacterium diphtheriae*
 - C. *Leuconostoc* sp.
 - D. *Streptococcus agalactiae*

16. On Tinsdale agar, colonies of *Corynebacterium diphtheriae* are characterized by the observance of
 - A. Liquefaction of the agar surrounding the colonies on the medium
 - B. Opalescent colonies with a white precipitate in the surrounding agar
 - C. Black colonies on the culture medium surrounded by brown halos
 - D. Pitting of the agar medium surrounding the colonies
17. Precipitates of diphtheria toxin and antitoxin formed in agar gels are an *in vitro* means for detecting toxigenic strains of *Corynebacterium diphtheriae*. The name of this test procedure is the
 - A. D-test
 - B. Elek test
 - C. Hodge test
 - D. Nagler test
18. The etiologic agent of the disease erysipelas is
 - A. *Staphylococcus aureus*
 - B. *Streptobacillus moniliformis*
 - C. *Streptococcus agalactiae*
 - D. *Streptococcus pyogenes*
19. *Staphylococcus aureus*, when present, could most likely be recovered from a stool sample if the primary plating medium included
 - A. Bismuth sulfite
 - B. Phenylethyl alcohol
 - C. Thiosulfate citrate bile salts sucrose
 - D. Xylose-lysine-desoxycholate
20. A common member of the normal flora of the upper respiratory tract is
 - A. *Corynebacterium jeikeium*
 - B. *Lactobacillus*
 - C. *Staphylococcus epidermidis*
 - D. Viridans streptococcus
21. Streptococci obtain all their energy from the fermentation of sugars to
 - A. Formic acid
 - B. Lactic acid
 - C. Succinic acid
 - D. Valeric acid
22. Streptococci are unable to synthesize the enzyme
 - A. Catalase
 - B. Kinase
 - C. Hyaluronidase
 - D. Lipase
23. The beta-hemolysis produced by group A *Streptococcus* seen on the surface of a sheep blood agar plate is primarily the result of streptolysin
 - A. H
 - B. M
 - C. O
 - D. S
24. When an infection due to *Streptomyces* is suspected, isolates can be separated from most other bacteria by
 - A. Heat shocking the culture
 - B. Incubating the culture at 25°C
 - C. Incubating the culture at greater than 37°C
 - D. Drying the specimen before inoculating the culture media
25. The production of H₂S is one characteristic used to differentiate which of the aerobic gram-positive bacilli?
 - A. *Corynebacterium*
 - B. *Erysipelothrix*
 - C. *Lactobacillus*
 - D. *Nocardia*

26. Growth in a 48-hour semisolid agar stab culture at room temperature reveals lateral filamentous growth away from the stab near the top of the medium. This observation is most characteristic of which organism?
 - A. *Rhodococcus* sp.
 - B. *Corynebacterium urealyticum*
 - C. *Enterococcus faecalis*
 - D. *Listeria monocytogenes*
27. A former species of *Corynebacterium* pathogenic for swine, horses, and cattle is also known to cause disease in compromised hosts. This organism when grown on culture media produces pale pink colonies that help to presumptively identify it as
 - A. *Arcanobacterium hemolyticum*
 - B. *Actinomyces naeslundii*
 - C. *Gardnerella vaginalis*
 - D. *Rhodococcus equi*
28. Which one of the following is *not* appropriate when describing *Streptococcus pneumoniae*?
 - A. Bile-resistant
 - B. Alpha-hemolytic
 - C. Lancet-shaped, gram-positive diplococcus
 - D. Virulent strains are encapsulated.
29. *Nocardia* can be differentiated from *Actinomyces* based on
 - A. *Nocardia* being an obligate anaerobe
 - B. The partial-acid fast staining reaction of *Actinomyces*
 - C. The production of sulfur granules in cases of nocardiosis
 - D. *Nocardia* being catalase positive
30. *Enterococcus faecium* is characteristically
 - A. Inhibited by the presence of bile in culture media
 - B. Able to grow in the presence of high concentrations of salt
 - C. PYR negative
 - D. Beta-hemolytic
31. A negative PYR (L-pyrolidonyl- α -naphthylamide) test is demonstrated by
 - A. *Enterococcus faecalis*
 - B. *Enterococcus faecium*
 - C. *Streptococcus pyogenes*
 - D. Viridans streptococci
32. A Gram stain of a sputum specimen from a patient with a suspected case of lobar pneumonia reveals many white blood cells and many gram-positive cocci, which are primarily diplococci. Which of the following statements would be appropriate, given these findings?
 - A. A PYR test should be performed on the culture isolate.
 - B. An Elek test should be performed on the culture isolate.
 - C. An optochin test should be performed on the culture isolate.
 - D. A hippurate hydrolysis test should be performed on the culture isolate.
33. A child presented in August at the pediatric clinic with a superficial skin infection of the neck. The large, itchy lesions were cultured, and the diagnosis of impetigo was made. One of the etiologic agents of this clinical condition is
 - A. *Erysipelothrix rhusiopathiae*
 - B. *Corynebacterium diphtheriae*
 - C. *Staphylococcus saprophyticus*
 - D. *Streptococcus pyogenes*

34. An identifying characteristic of *Staphylococcus aureus* is
A. DNase negative
B. Coagulase negative
C. Mannitol fermentation positive
D. Growth inhibition in presence of increased salt
35. Which of the following organisms is able to hydrolyze sodium hippurate to benzoic acid and glycine?
A. *Streptococcus agalactiae*
B. *Streptococcus pneumoniae*
C. *Listeria monocytogenes*
D. *Enterococcus faecalis*
36. Which of the following is *not* characteristic of *Listeria monocytogenes*?
A. CAMP test positive
B. Catalase negative
C. Esculin hydrolysis positive
D. Motile
37. Which of the following is *not* associated with *Staphylococcus aureus*?
A. Endotoxin production
B. Clumping factor production
C. Deoxyribonuclease production
D. Hemolysin production
38. Which of the following is a characteristic of staphylococci that would help in their isolation from clinical specimens?
A. Bile resistance
B. Growth at 55°C
C. High salt tolerance
D. Resistance to novobiocin
39. Which of the following species of *Bacillus* is nonmotile?
A. *B. cereus*
B. *B. subtilis*
C. *B. anthracis*
D. *B. thuringiensis*
40. Which one of the following diseases involves erythrogenic toxin?
A. Cutaneous anthrax
B. Diphtheria
C. Impetigo
D. Scarlet fever
41. Cultures of the posterior pharynx are most commonly submitted to the clinical laboratory for the detection of
A. *Corynebacterium diphtheriae*
B. *Staphylococcus aureus*
C. *Streptococcus pneumoniae*
D. *Streptococcus pyogenes*
42. *Streptococcus sanguis*, a viridans streptococcus, is most commonly associated with which of the following clinical conditions?
A. Otitis media
B. Pharyngitis
C. Relapsing fever
D. Subacute bacterial endocarditis
43. Rust-colored sputum in cases of lobar pneumonia is characteristic of which of the following possible etiologic agents?
A. *Corynebacterium jeikeium*
B. *Staphylococcus aureus*
C. *Streptococcus pneumoniae*
D. *Streptococcus pyogenes*
44. A urine culture from a 23-year-old female grew a catalase-positive gram-positive coccus (>100,000 cfu/mL), which would most likely be
A. *Staphylococcus saprophyticus*
B. *Enterococcus faecalis*
C. *Streptococcus bovis* group
D. *Streptococcus viridans*

45. Cystine-tellurite blood agar plates are recommended for the isolation of
 - A. *Corynebacterium diphtheriae*
 - B. *Streptococcus agalactiae*
 - C. *Streptococcus pyogenes*
 - D. Group D streptococci
46. The pulmonary form of anthrax is known as
 - A. Valley fever
 - B. Walking pneumonia
 - C. Farmers' lung
 - D. Woollsorters disease
47. Pleomorphic gram-positive bacilli in a Gram stain best describes
 - A. *Bacillus anthracis*
 - B. *Bacillus subtilis*
 - C. *Listeria monocytogenes*
 - D. *Corynebacterium pseudodiphtheriticum*
48. An aerobic gram-positive rod known to cause bacteremia in hospitalized immunocompromised patients is
 - A. *Bacillus anthracis*
 - B. *Corynebacterium jeikeium*
 - C. *Corynebacterium ulcerans*
 - D. *Corynebacterium urealyticum*
49. A bone marrow transplant patient on immunosuppressive therapy developed a pulmonary abscess with symptoms of neurologic involvement. A brain abscess was detected by MRI, and aspirated material grew an aerobic, filamentous, branching gram-positive organism, which stained weakly acid-fast. The most likely etiologic agent in this case would be
 - A. *Actinomyces israelii*
 - B. *Nocardia asteroides*
 - C. *Mycobacterium tuberculosis*
 - D. *Propionibacterium acnes*
50. Which of the following is catalase negative?
 - A. *Bacillus*
 - B. *Corynebacterium*
 - C. *Leuconostoc*
 - D. *Listeria*
51. Colonies of *Listeria monocytogenes* on a sheep blood agar plate most closely resemble colonies of
 - A. *Corynebacterium diphtheriae*
 - B. *Streptococcus agalactiae*
 - C. *Streptococcus bovis* group
 - D. *Rhodococcus equi*
52. The most common etiologic agent of infections associated with the surgical insertion of prosthetic devices such as artificial heart valves and cerebrospinal fluid shunts is
 - A. *Corynebacterium urealyticum*
 - B. *Staphylococcus capitis*
 - C. *Staphylococcus epidermidis*
 - D. *Streptococcus mutans*
53. The description of "Medusa head" colonies on solid agar is most characteristic of
 - A. *Bacillus anthracis*
 - B. *Enterococcus faecalis*
 - C. *Staphylococcus saprophyticus*
 - D. *Streptococcus agalactiae*
54. Which of the following is most likely to be isolated in cultures from the anterior nares of healthcare workers?
 - A. *Bacillus cereus*
 - B. *Streptococcus pneumoniae*
 - C. *Staphylococcus aureus*
 - D. *Staphylococcus saprophyticus*

55. Ethylhydrocupreine HCl susceptibility is a presumptive test for the identification of
 - A. Viridans streptococci
 - B. *Streptococcus pyogenes*
 - C. *Streptococcus agalactiae*
 - D. *Streptococcus pneumoniae*
56. Solubility in the presence of sodium desoxycholate is characteristic of
 - A. *Enterococcus faecalis*
 - B. *Streptococcus agalactiae*
 - C. *Streptococcus mutans*
 - D. *Streptococcus pneumoniae*
57. Family members attending a picnic became ill about 2 hours after eating. The illness was characterized by rapid onset of violent vomiting. The most likely bacterial cause of such symptoms would be food poisoning caused by
 - A. *Enterococcus faecium*
 - B. *Bacillus subtilis*
 - C. *Listeria monocytogenes*
 - D. *Staphylococcus aureus*
58. The novobiocin susceptibility test is used for the identification of
 - A. *Corynebacterium diphtheriae*
 - B. *Streptococcus pyogenes*
 - C. *Streptococcus pneumoniae*
 - D. *Staphylococcus saprophyticus*
59. Tellurite reduction is used for the presumptive identification of
 - A. *Bacillus anthracis*
 - B. *Corynebacterium diphtheriae*
 - C. *Erysipelothrix rhusiopathiae*
 - D. *Staphylococcus saprophyticus*
60. The etiologic agent of the majority of adult joint infections is
 - A. *Abiotrophia* sp.
 - B. *Leuconostoc* sp.
 - C. *Staphylococcus aureus*
 - D. *Streptococcus pneumoniae*
61. Which of the following is associated with infections in humans often linked to deli meats and improperly pasteurized dairy products?
 - A. *Bacillus subtilis*
 - B. *Listeria monocytogenes*
 - C. *Leuconostoc*
 - D. *Streptococcus agalactiae*
62. *Bacillus cereus* has been implicated as the etiologic agent in cases of
 - A. Food poisoning
 - B. Impetigo
 - C. Pelvic inflammatory disease
 - D. Toxic shock syndrome
63. The causative agent of “malignant pustule” is
 - A. *Bacillus anthracis*
 - B. *Corynebacterium ulcerans*
 - C. *Erysipelothrix rhusiopathiae*
 - D. *Listeria monocytogenes*
64. An infant was hospitalized with a severe, tender erythema. The child’s epidermis was loose, and large areas of skin could be peeled off. The condition described is most consistent with a clinical syndrome associated with
 - A. *Streptococcus pyogenes*
 - B. *Staphylococcus aureus*
 - C. *Bacillus anthracis*
 - D. *Erysipelothrix rhusiopathiae*
65. A catalase-negative gram-positive coccus is isolated from a urine sample of a hospitalized patient. The bacterium produced a black pigment on bile-esculin agar and formed acid from glucose in the presence of 6.5% NaCl. What is the most likely identification of this bacterium?
 - A. *Abiotrophia* sp.
 - B. *Enterococcus faecalis*
 - C. Group B streptococci
 - D. Group D streptococci

Aerobic Gram-Negative Bacteria

66. In suspected cases of brucellosis, the optimal specimen to be collected for the isolation of the etiologic agent is
 - A. Blood
 - B. Urine
 - C. Cerebrospinal fluid
 - D. Nasopharyngeal exudates
67. The majority of clinical isolates of *Klebsiella* are
 - A. *K. ozaenae*
 - B. *K. pneumoniae*
 - C. *K. aerogenes*
 - D. *K. oxytoca*
68. The enterotoxins of both *Vibrio cholerae* O1 and noninvasive (toxigenic) strains of *Escherichia coli* produce serious diarrhea by what mechanism?
 - A. Stimulation of adenylate cyclase, which gives rise to excessive fluid secretion by the cells of the small intestine
 - B. Penetration of the bowel mucosa
 - C. Stimulation of colicin production
 - D. Elaboration of a dermonecrotizing toxin
69. Colonies of *Neisseria* sp. turn color when a redox reagent is applied. The color change is indicative of the activity of the bacterial enzyme
 - A. Beta-galactosidase
 - B. Urease
 - C. Cytochrome oxidase
 - D. Phenylalanine deaminase
70. Which of the following is *not* true of *Shigella sonnei*?
 - A. Large numbers of organisms must be ingested to produce disease.
 - B. The organism produces an inflammatory condition in the large intestine with bloody diarrhea.
 - C. The organism produces disease most commonly in the pediatric population.
 - D. The organism is a delayed lactose fermenter.
71. An environmental sampling study of respiratory therapy equipment produced cultures of a yellow, nonfermentative (at 48 hours), gram-negative bacillus from several of the nebulizers, which would most likely be species of
 - A. *Chryseobacterium*
 - B. *Pseudomonas*
 - C. *Alcaligenes*
 - D. *Moraxella*
72. The characteristics of being lactose negative, citrate negative, urease negative, lysine decarboxylase negative, and nonmotile best describe which organism?
 - A. *Proteus vulgaris*
 - B. *Yersinia pestis*
 - C. *Salmonella enterica*
 - D. *Shigella dysenteriae*
73. A fermentative gram-negative bacillus that is oxidase positive, motile, and grows well on MacConkey agar is
 - A. *Aeromonas hydrophila*
 - B. *Pseudomonas aeruginosa*
 - C. *Stenotrophomonas maltophilia*
 - D. *Yersinia enterocolitica*

74. Fecal cultures are inoculated on thiosulfate-citrate-bile salts-sucrose agar specifically for the isolation of
 A. *Shigella*
 B. *Vibrio*
 C. *Campylobacter*
 D. *Salmonella*
75. The K antigen of the family *Enterobacteriaceae* is
 A. Heat labile
 B. The somatic antigen
 C. Located on the flagellum
 D. The antigen used to group *Shigella*
76. The causative agent of melioidosis is
 A. *Burkholderia cepacia*
 B. *Burkholderia pseudomallei*
 C. *Moraxella catarrhalis*
 D. *Stenotrophomonas maltophilia*
77. Which microorganism will grow only on culture media supplemented with either cysteine or cystine?
 A. *Actinobacillus lignieresii*
 B. *Bartonella bacilliformis*
 C. *Francisella tularensis*
 D. *Kingella kingae*

78. A culture of a decubitus ulcer grew a gram-negative facultative bacillus. On TSI it produced an acid slant, acid butt, and gas. Test reactions in other media were as follows:

Citrate	negative
Indole	positive
Urease	negative
ONPG	positive
Voges-Proskauer	negative

The organism was identified as

- A. *Enterobacter cloacae*
 B. *Escherichia coli*
 C. *Citrobacter (diversus) koseri*
 D. *Providencia stuartii*
79. An example of an oxidase-positive, glucose nonfermenting organism is
 A. *Aeromonas hydrophila*
 B. *Escherichia coli*
 C. *Klebsiella pneumoniae*
 D. *Pseudomonas aeruginosa*
80. A fastidious gram-negative bacillus was isolated from a case of periodontal disease, which upon darkfield examination was noted to have gliding motility. The most likely identification of this etiologic agent would be
 A. *Capnocytophaga*
 B. *Chromobacterium*
 C. *Kingella*
 D. *Plesiomonas*

81. The species of *Vibrio* closely associated with rapidly progressing wound infections seen in patients with underlying liver disease is
 - A. *V. alginolyticus*
 - B. *V. cholerae*
 - C. *V. vulnificus*
 - D. *V. parahaemolyticus*
82. Severe disseminated intravascular coagulation often complicates cases of septicemia caused by
 - A. *Acinetobacter* sp.
 - B. *Moraxella* sp.
 - C. *Neisseria gonorrhoeae*
 - D. *Neisseria meningitidis*
83. The *Haemophilus influenzae* vaccine protects against which serotype?
 - A. Serotype a
 - B. Serotype b
 - C. Serotype c
 - D. Serotype d
84. *Salmonella* Typhi exhibits a characteristic biochemical pattern, which differentiates it from the other salmonellae. Which of the following is *not* characteristic of *S. Typhi*?
 - A. Large amounts of H_2S are produced in TSI agar.
 - B. Agglutination in Vi grouping serum
 - C. Lysine decarboxylase positive
 - D. Citrate negative
85. The sexually acquired disease characterized by genital ulcers and tender inguinal lymphadenopathy, which is caused by a small, gram-negative bacillus, is known as
 - A. Chancroid
 - B. Bacterial vaginosis
 - C. Syphilis
 - D. Trachoma
86. Which of the following diseases is most likely to be acquired from a hot tub or whirlpool?
 - A. Q fever
 - B. Erysipelas
 - C. *Acinetobacter* cellulitis
 - D. *Pseudomonas* dermatitis
87. *Campylobacter* spp. are associated most frequently with cases of
 - A. Osteomyelitis
 - B. Gastroenteritis
 - C. Endocarditis
 - D. Appendicitis
88. An organism occasionally misidentified as an enteric pathogen because it produces a large amount of H_2S is
 - A. *Burkholderia cepacia*
 - B. *Burkholderia pseudomallei*
 - C. *Pseudomonas putida*
 - D. *Shewanella putrefaciens*
89. The etiologic agent of whooping cough is
 - A. *Bordetella pertussis*
 - B. *Brucella suis*
 - C. *Francisella tularensis*
 - D. *Haemophilus ducreyi*
90. An important characteristic of *Neisseria gonorrhoeae* or the infection it produces is
 - A. A Gram stain of the organism reveals gram-negative bacilli.
 - B. Asymptomatic infections are common in females.
 - C. Produces disease in humans and domestic animals
 - D. The bacteria survive long periods outside the host's body.
91. Which of the following organisms would most likely produce the biochemical reactions shown in Color Plate 27■?
 - A. *Citrobacter freundii*
 - B. *Proteus mirabilis*
 - C. *Providencia rettgeri*
 - D. *Salmonella*

92. A gram-negative, “kidney bean” cellular morphology is a distinguishing characteristic of
A. *Neisseria meningitidis*
B. *Yersinia pestis*
C. *Bartonella* spp.
D. *Actinobacter* spp.
93. Which of the following nonfermenters is rarely isolated in the U.S.?
A. *Pseudomonas aeruginosa*
B. *Stenotrophomonas maltophilia*
C. *Burkholderia mallei*
D. *Burkholderia cepacia*
94. Erythromycin eye drops are routinely administered to infants to prevent infections by
A. *E. coli*
B. *Haemophilus influenzae*
C. *Pseudomonas aeruginosa*
D. *Neisseria gonorrhoeae*
95. *Neisseria lactamica* closely resembles *Neisseria meningitidis* but can be differentiated from it by its ability to metabolize
A. Maltose
B. Lactose
C. Glucose
D. Sucrose
96. A causative agent of the form of conjunctivitis known as pinkeye is
A. *Haemophilus aegyptius*
B. *Moraxella lacunata*
C. *Chlamydia trachomatis*
D. *Klebsiella ozaenae*
97. The single species in the genus *Hafnia* is
A. *alvei*
B. *gergoviae*
C. *ruckeri*
D. *tarda*
98. *Acinetobacter baumannii*
A. Requires cysteine
B. Is oxidase negative
C. Ferments glucose
D. Does not grow on MacConkey agar
99. *Legionella pneumophila* is the etiologic agent of both Legionnaires disease and
A. Swine fever
B. Pontiac fever
C. Rift Valley fever
D. San Joaquin Valley fever
100. In suspected cases of brucellosis, what is the most sensitive specimen to submit for culture?
A. Bone marrow
B. Nasopharyngeal swab
C. Sputum
D. Stool
101. Hemolytic uremic syndrome is a complication after infection with
A. *E. coli* O157:H7
B. *Salmonella* Typhi
C. *Vibrio cholerae* O1
D. *Yersinia enterocolitica*
102. Identify the fermentative agent that may infect reptiles and fish as well as humans when they are exposed to contaminated soil or water.
A. *Aeromonas*
B. *Chromobacterium*
C. *Chryseobacterium*
D. *Enterobacter*
103. *Campylobacter jejuni* is
A. Nonmotile
B. Oxidase negative
C. Hippurate hydrolysis positive
D. A straight gram-negative bacillus

104. Which of the following has a negative oxidase test?
 - A. *Aeromonas*
 - B. *Chryseobacterium*
 - C. *Hafnia*
 - D. *Vibrio*
105. Which of the following is *true* of *Neisseria gonorrhoeae*?
 - A. Adversely affected by fatty acids in clinical specimens
 - B. Rapid growth on sheep blood agar
 - C. Ferments glucose and maltose
 - D. ONPG positive
106. The method of serogrouping *Shigella* used in the clinical laboratory is based on
 - A. Bacteriocins
 - B. H antigens
 - C. K antigens
 - D. O antigens
107. The symptom of diffuse, watery diarrhea that produces a relatively clear stool containing mucus flecks is suggestive of an infection caused by
 - A. Enterohemorrhagic *Escherichia coli*
 - B. *Shigella dysenteriae*
 - C. *Vibrio cholerae*
 - D. *Yersinia enterocolitica*
108. An example of a halophilic micro-organism is
 - A. *Morganella morganii*
 - B. *Plesiomonas shigelloides*
 - C. *Vibrio parahaemolyticus*
 - D. *Yersinia pestis*
109. Which of the following statements is *not* true of *Brucella*?
 - A. Infection may occur via abrasions of the oral mucosa, conjunctiva, and genitals.
 - B. They are fastidious and require supplemented media for isolation.
 - C. The risk of accidental laboratory infection is no greater than with any other organism.
 - D. Phage and dye sensitivity tests are used for identification to the species level.
110. Infection of the gastric mucosa leading to gastritis or peptic ulcers is most commonly associated with
 - A. *Campylobacter jejuni*
 - B. *Helicobacter pylori*
 - C. *Salmonella* Typhi
 - D. *Shigella sonnei*
111. Which of the the following does *not* describe *Acinetobacter* sp.?
 - A. Commonly susceptible to most antimicrobials
 - B. Generally coccobacillary in morphology
 - C. Oxidase negative
 - D. Infections associated with use of medical devices
112. Explosive watery diarrhea with severe abdominal pain after eating raw shellfish is most characteristic of infection caused by
 - A. *Campylobacter jejuni*
 - B. *Helicobacter pylori*
 - C. *Shigella dysenteriae*
 - D. *Vibrio parahemolyticus*
113. An unheated suspension of *Salmonella* Typhi typically produces agglutination of Vi antisera. After heating the same suspension, agglutination will occur in which grouping sera?
 - A. A
 - B. B
 - C. C₁
 - D. D

114. The species of *Campylobacter* noted to produce septicemia, septic arthritis, meningitis, jaundice with hepatomegaly, and thrombophlebitis in debilitated patients is
 - A. *C. coli*
 - B. *C. fetus*
 - C. *C. laris*
 - D. *C. sputorum*
115. *Shigella sonnei* is differentiated from other *Shigella* sp. by
 - A. A positive ONPG
 - B. Its positive phenylalanine deaminase reaction
 - C. Its negative oxidase reaction
 - D. Its ability to demonstrate motility at 22°C
116. A positive Voges-Proskauer reaction is characteristic of
 - A. *Enterobacter aerogenes*
 - B. *Escherichia coli*
 - C. *Proteus vulgaris*
 - D. *Providencia rettgeri*
117. Which of the following is *not* true regarding virulent strains of *Vibrio cholerae*?
 - A. Adherent to enterocytes
 - B. Mucinase production
 - C. Nonmotile
 - D. Toxigenic
118. The classic toxigenic strains of which serogroup are implicated in epidemic infections of *Vibrio cholerae*?
 - A. O1
 - B. O2
 - C. O3
 - D. O4
119. *Brucella* spp. are
 - A. The etiologic agents of relapsing fever
 - B. Small spiral organisms
 - C. Primarily a cause of endogenous human infections
 - D. Intracellular pathogens
120. Which of the following reactions is typical for *Escherichia coli*?
 - A. Beta-hemolytic on sheep blood agar
 - B. Colorless colonies on MacConkey agar
 - C. Colorless colonies on xylose-lysine-desoxycholate agar
 - D. Green colonies with black centers on Hektoen enteric agar
121. *Yersinia pestis* is characteristically
 - A. Urease negative
 - B. Hydrogen sulfide positive
 - C. Motile at 20–25°C
 - D. Oxidase positive
122. Lack of motility is characteristic of
 - A. *Enterobacter cloacae*
 - B. *Klebsiella oxytoca*
 - C. *Morganella morganii*
 - D. *Providencia stuartii*
123. In cases of legionellosis
 - A. Person-to-person transmission is common
 - B. Farm animals are important animal reservoirs
 - C. Patients can present with a self-limited nonpneumonic febrile illness
 - D. Specimens may be cold enriched to enhance recovery of the organism
124. Which of the following is *not* a characteristic of *Klebsiella (Calymmatobacterium) granulomatis*?
 - A. Is often sexually transmitted
 - B. Is isolated on chocolate agar
 - C. Is the causative agent of granuloma inguinale
 - D. Can be diagnosed by detection of Donovan bodies in clinical specimens

125. A positive DNase would be seen with
 - A. *Escherichia coli*
 - B. *Klebsiella oxytoca*
 - C. *Proteus mirabilis*
 - D. *Serratia marcescens*
126. A negative citrate reaction is characteristic of
 - A. *Citrobacter freundii*
 - B. *Enterobacter aerogenes*
 - C. *Serratia marcescens*
 - D. *Shigella boydii*
127. Isolation of *Neisseria gonorrhoeae*
 - A. Is enhanced by cold enrichment
 - B. Requires incubation under increased CO₂
 - C. From contaminated sites is made easier by the use of CIN agar
 - D. Is not affected if clinical specimen is refrigerated before culturing
128. A positive indole reaction is characteristic of
 - A. *Escherichia coli*
 - B. *Proteus mirabilis*
 - C. *Salmonella Choleraesuis*
 - D. *Serratia marcescens*
129. Which one of the following organisms would produce a yellow slant and a yellow butt on TSI agar after incubating 18 hours?
 - A. *Escherichia coli*
 - B. *Proteus mirabilis*
 - C. *Salmonella Typhimurium*
 - D. *Shigella sonnei*
130. Pyocyanin is characteristically produced by
 - A. *Pseudomonas aeruginosa*
 - B. *Pseudomonas fluorescens*
 - C. *Shewanella putrefaciens*
 - D. *Stenotrophomonas maltophilia*
131. Color Plate 28■ shows the Gram stain of cerebrospinal fluid from a 1-year-old girl suspected of having meningitis. After 24 hours of growth, small tan colonies were isolated on chocolate agar incubated in CO₂. Sheep blood agar also incubated in CO₂ had no growth. Which of the following organisms should be suspected?
 - A. *Brucella canis*
 - B. *Bordetella parapertussis*
 - C. *Haemophilus influenzae*
 - D. *Neisseria meningitidis*
132. Which of the following is *true* concerning *Campylobacter jejuni*?
 - A. Catalase negative
 - B. Isolated best at 24°C
 - C. Hydrogen sulfide positive
 - D. A leading cause of bacterial diarrhea worldwide
133. Of the following microorganisms, which one will turn a dark purple when tetramethyl-*p*-phenylenediamine hydrochloride is applied?
 - A. *Acinetobacter baumannii*
 - B. *Stenotrophomonas maltophilia*
 - C. *Moraxella catarrhalis*
 - D. *Yersinia enterocolitica*
134. *Cardiobacterium hominis*, an inhabitant of the upper respiratory tract of humans, has been recovered as the etiologic agent from cases of endocarditis. An identifying characteristic of the organism is
 - A. Positive oxidase
 - B. Positive catalase
 - C. Indole negative
 - D. Inability to grow on sheep blood agar
135. *Vibrio vulnificus* is a well-established human pathogen that is known to cause
 - A. Gastroenteritis
 - B. Pneumonia
 - C. Pyelonephritis
 - D. Wound infections

136. Which of the following organisms produce a positive phenylalanine deaminase reaction?
 - A. *Citrobacter freundii*
 - B. *Klebsiella pneumoniae*
 - C. *Providencia stuartii*
 - D. *Yersinia enterocolitica*
137. Which of the following non-lactose-fermenting organisms does *not* produce fluorescein?
 - A. *Pseudomonas alcaligenes*
 - B. *Pseudomonas aeruginosa*
 - C. *Pseudomonas fluorescens*
 - D. *Pseudomonas putida*
138. Which of the following organisms is unable to grow on MacConkey agar?
 - A. *Bordetella bronchiseptica*
 - B. *Burkholderia cepacia*
 - C. *Kingella denitrificans*
 - D. *Plesiomonas shigelloides*
139. Which of the the following is *not* true of *Haemophilus* spp.?
 - A. Obligate parasites
 - B. Grow well on sheep blood agar
 - C. Small, pleomorphic, gram-negative coccobacilli
 - D. Many are found as normal flora in the human respiratory tract.
140. *Legionella pneumophila*
 - A. Infections are most often acquired from environmental sources
 - B. Metabolizes a number of carbohydrates
 - C. Stains easily on the routine Gram stain
 - D. Does not autofluoresce
141. Which of the following is the optimal clinical specimen for the recovery of *Legionella pneumophila*?
 - A. Stool
 - B. Blood
 - C. Bronchial washings
 - D. Nasopharyngeal swab
142. Which of the following is *not* an appropriate medium for primary isolation of *Bordetella pertussis*?
 - A. Regan-Lowe
 - B. Bordet-Gengou
 - C. Modified Skirrow's medium
 - D. Buffered charcoal yeast extract
143. Production of a yellow pigment is characteristic of which of the following *Enterobacter* sp.?
 - A. *E. aerogenes*
 - B. *E. cloacae*
 - C. *E. sakazakii*
 - D. *E. taylora*
144. A gram-negative bacillus was recovered from the urine of a child with a history of recurrent urinary tract infections. The organism was oxidase negative, lactose negative, urease positive, and motile. The most likely identification of this agent would be
 - A. *Escherichia coli*
 - B. *Klebsiella pneumoniae*
 - C. *Proteus mirabilis*
 - D. *Pseudomonas aeruginosa*
145. *Acinetobacter baumannii* is characteristically
 - A. Motile
 - B. Oxidase positive
 - C. Sensitive to penicillin
 - D. Able to grow on MacConkey agar
146. Which of the following is *not* characteristic of *Eikenella corrodens*?
 - A. It is a thin, gram-negative bacillus.
 - B. Colonies sometimes pit the surface of the agar medium.
 - C. It is found in the mouth and upper respiratory tract of humans.
 - D. It is often found in pure culture when recovered from infections.

147. Which of the following species of *Pasteurella* is associated with human infections following cat bites?
 - A. *P. avium*
 - B. *P. canis*
 - C. *P. multocida*
 - D. *P. stomatis*
148. Which of the following *Legionella* spp. is positive for hippurate hydrolysis?
 - A. *L. micdadei*
 - B. *L. longbeachae*
 - C. *L. gormanii*
 - D. *L. pneumophila*
149. Which of the following is *not* characteristic of *Haemophilus influenzae* biogroup *aegyptius*?
 - A. Indole negative
 - B. Requires V factor
 - C. Requires X factor
 - D. Aminolevulinic acid positive
150. *Kingella denitrificans* can be differentiated from *Neisseria gonorrhoeae* because it is
 - A. Able to grow on Thayer-Martin agar
 - B. Able to reduce nitrates
 - C. Oxidase positive
 - D. Glucose positive
151. *Plesiomonas* was recently moved to which family?
 - A. *Brucellaceae*
 - B. *Enterobacteriaceae*
 - C. *Legionellaceae*
 - D. *Vibrionaceae*
152. In the past, povidone iodine, tincture of iodine prep pads and swabs, and other disinfectants have been recalled because of microbial contamination. The most likely organism to be isolated in these cases is
 - A. *Bordetella bronchiseptica*
 - B. *Klebsiella pneumoniae*
 - C. *Pseudomonas aeruginosa*
 - D. *Serratia marcescens*
153. Foodborne outbreaks of brucellosis are most commonly associated with eating
 - A. Raw shellfish
 - B. Imported cheese
 - C. Contaminated potato salad
 - D. Improperly cooked hamburger
154. *Serratia* spp. are unique in the family *Enterobacteriaceae* because of their ability to produce extracellular hydrolytic enzymes. Which of the following is *not* produced by *Serratia* species?
 - A. DNase
 - B. Gelatinase
 - C. Lipase
 - D. NADase
155. *Haemophilus ducreyi* is the causative agent of
 - A. Chancroid
 - B. Lymphogranuloma venereum
 - C. Trachoma
 - D. Whooping cough
156. The optimal specimen for the recovery of *Bordetella pertussis* is
 - A. Anterior nares swab
 - B. Blood
 - C. Expecterated sputum
 - D. Nasopharyngeal swab

157. Which of the following is the most common indicator of bacterial vaginosis?
A. *Eikenella*
B. *Capnocytophaga*
C. *Gardnerella*
D. *Listeria*
158. Blood cultures are recommended for the recovery of which of the following microorganisms?
A. *Acinetobacter baumannii*
B. *Brucella canis*
C. *Chlamydia trachomatis*
D. *Yersinia enterocolitica*
159. *Yersinia pseudotuberculosis* is known to manifest commonly as which of the following clinical conditions?
A. Epiglottitis
B. Hepatitis
C. Mesenteric lymphadenitis
D. Pseudomembranous colitis
160. Cefsulodin-irgasan-novobiocin (CIN) agar is recommended for the recovery of
A. *Brucella suis*
B. *Cardiobacterium hominis*
C. *Rhodococcus equi*
D. *Yersinia enterocolitica*
161. Why are cultures for *Gardnerella vaginalis* not recommended?
A. The bacteria grow so slowly that results take too long to be relevant.
B. Isolation of the bacteria may not be clinically significant.
C. It is unsafe to grow this bacterium.
D. Artificial media are not available.
162. Buffered charcoal yeast extract agar is the recommended medium for the recovery of
A. *Hafnia alvei*
B. *Legionella pneumophila*
C. *Neisseria meningitidis*
D. *Vibrio cholerae*
163. Swimmer's ear, a form of external otitis is commonly caused by
A. *Acinetobacter baumannii*
B. *Bordetella bronchiseptica*
C. *Haemophilus influenzae*
D. *Pseudomonas aeruginosa*
164. A number of vacationers who have traveled outside the U.S. have had their vacations interrupted by a case of "traveler's diarrhea," which is commonly associated with which etiologic agent?
A. *Aeromonas hydrophila*
B. *Escherichia coli*
C. *Proteus mirabilis*
D. *Vibrio parahemolyticus*
165. On Gram stain, a morphology that resembles "seagull wings" is most characteristic of
A. *Campylobacter jejuni*
B. *Neisseria gonorrhoeae*
C. *Plesiomonas shigelloides*
D. *Yersinia pseudotuberculosis*
166. The flattened adjacent sides of the cellular appearance of which microorganism are said to resemble kidney beans?
A. *Aeromonas hydrophila*
B. *Campylobacter jejuni*
C. *Neisseria gonorrhoeae*
D. *Pasteurella multocida*
167. Which of the following is *not* urease positive?
A. *Salmonella*
B. *Helicobacter pylori*
C. *Proteus mirabilis*
D. *Yersinia enterocolitica*
168. Which of the *Neisseria* spp. produces acid from glucose but not from maltose, lactose, or sucrose?
A. *Neisseria gonorrhoeae*
B. *Neisseria lactamica*
C. *Neisseria meningitidis*
D. *Neisseria sicca*

169. Which of the *Neisseria* spp. produces acid from glucose and maltose but not sucrose or lactose?
- Neisseria gonorrhoeae*
 - Neisseria lactamica*
 - Neisseria meningitidis*
 - Neisseria sicca*
170. The causative agent of the septicemic, hemolytic disease known as Oroya fever is
- Bartonella bacilliformis*
 - Burkholderia mallei*
 - Haemophilus aegyptius*
 - Yersinia pestis*
171. A positive gelatin reaction is characteristic of
- Morganella morganii*
 - Proteus vulgaris*
 - Salmonella Typhimurium*
 - Serratia liquefaciens*
172. A positive phenylalanine deaminase reaction is characteristic of
- Hafnia alvei*
 - Moraxella catarrhalis*
 - Proteus vulgaris*
 - Salmonella Enteritidis*
173. Lack of motility is a characteristic of
- Enterobacter*
 - Klebsiella*
 - Salmonella*
 - Serratia*
174. Violet-colored colonies are typically produced by
- Chromobacterium violaceum*
 - Chryseobacterium meningosepticum*
 - Pseudomonas aeruginosa*
 - Serratia marcescens*
175. Which of the following is *not* true about *Pasteurella multocida*?
- Most common human infections occur in soft tissues, bones, and joints.
 - Humans harbor the organism as part of their normal flora.
 - It is the most virulent of the species in the genus.
 - It grows on sheep blood agar.
176. Pus was aspirated from an empyema. A Gram stain of the aspirated material showed many white blood cells and numerous gram-negative bacilli. The culture grew many colonies producing a soluble green pigment. The most likely etiologic agent in this case would be
- Chromobacterium violaceum*
 - Legionella pneumophila*
 - Pseudomonas aeruginosa*
 - Serratia marcescens*
177. Some strains of *Serratia marcescens* produce a red-colored pigment. Pigment production is enhanced by
- Incubation at 22°C
 - Incubation at 42°C
 - Growth on sheep blood agar
 - Growth on tryptic soy agar without blood
178. Which of the following is *not* a correct description regarding *Aeromonas hydrophila*?
- Beta-hemolytic
 - Catalase positive
 - ONPG negative
 - Oxidase positive
179. *Edwardsiella tarda* is occasionally isolated in stool specimens and can biochemically be confused with
- Salmonella*
 - Enterohemorrhagic *E. coli*
 - Vibrio cholerae*
 - Yersinia enterocolitica*

180. The porphyrin test determines an organism's requirement for
 - A. Cystiene
 - B. Hemin
 - C. NAD
 - D. Thiol
181. Which of the following is an occasional cause of respiratory tract infections and is rapidly urea positive?
 - A. *Bordetella bronchiseptica*
 - B. *Brucella abortus*
 - C. *Campylobacter fetus*
 - D. *Escherichia coli*
182. One of the most common etiologic agents of community-acquired uncomplicated cases of cystitis is
 - A. *Enterobacter aerogenes*
 - B. *Escherichia coli*
 - C. *Klebsiella pneumoniae*
 - D. *Proteus vulgaris*
183. The characteristic growth pattern known as "satelliting" is associated with
 - A. *Burkholderia pseudomallei*
 - B. *Campylobacter jejuni*
 - C. *Haemophilus influenzae*
 - D. *Yersinia pestis*
184. Colonies that are said to resemble "droplets of mercury" are characteristic of
 - A. *Bordetella pertussis*
 - B. *Burkholderia cepacia*
 - C. *Campylobacter jejuni*
 - D. *Yersinia pestis*
185. When an epidemiologic survey for the detection of upper respiratory tract carriers of *Neisseria meningitidis* or *Bordetella pertussis* is being conducted, the optimal type of specimen to be obtained for culture is
 - A. Anterior nares
 - B. Buccal cavity
 - C. Nasopharyngeal
 - D. Throat
186. Chronic carriers, persons who remain infected with an organism for long periods, are typically associated with the dissemination of
 - A. *Bordetella pertussis*
 - B. *Campylobacter jejuni*
 - C. *Salmonella* Typhi
 - D. *Yersinia pestis*
187. Milk has classically been the primary food associated with the transmission of some diseases, especially for those diseases of cattle transmissible to humans, such as
 - A. Brucellosis
 - B. Glanders
 - C. Melioidosis
 - D. Pontiac fever
188. Association with faucet aerators and humidifiers used with ventilators in intensive care units is commonly a factor in outbreaks of infections with which of the following microorganisms?
 - A. *Klebsiella pneumoniae*
 - B. *Pseudomonas aeruginosa*
 - C. *Salmonella* spp.
 - D. *Serratia marcescens*
189. Which of the following is *not* appropriate for the cultivation of *Neisseria gonorrhoeae*?
 - A. Chocolate agar
 - B. Cefsulodin-irgasan-novobiocin agar
 - C. Martin-Lewis agar
 - D. Modified Thayer-Martin agar
190. The selective nature of Hektoen enteric agar is due to the inclusion of which one of the following?
 - A. Bile salts
 - B. Bis-sodium metarsulfate
 - C. Bromthymol blue
 - D. NaCl

191. For the selective isolation of *Vibrio* spp. the recommended agar is
 - A. Thiosulfate-citrate-bile salt-sucrose agar
 - B. Charcoal yeast extract agar
 - C. Mannitol salt agar
 - D. Tinsdale agar
192. When performing the oxidase test, which of the following would *not* be appropriate?
 - A. The reagent used is *o*-nitrophenyl- β -D-galactopyranoside.
 - B. A nichrome wire loop should be used to acquire inoculum for testing.
 - C. Colonies from sheep blood agar can be used.
 - D. A positive colony turns dark purple within 10 seconds after application of the reagent.
193. The porphyrin test is most useful for the identification of which of the following?
 - A. *Campylobacter*
 - B. *Haemophilus*
 - C. *Moraxella*
 - D. *Neisseria*
194. Which of the following is *not* true about the laboratory diagnosis of pertussis?
 - A. Regan-Lowe medium is the recommended medium.
 - B. DFA test results are definitive and do not need to be confirmed by culture.
 - C. Calcium alginate or Dacron swabs are recommended over cotton-tipped swabs for specimen collection.
 - D. Material collected from the nasopharynx is optimal for detection of the causative agent.
195. Reptiles kept as pets are sometimes associated with the transmission of
 - A. *Campylobacter*
 - B. *Helicobacter*
 - C. *Salmonella*
 - D. *Vibrio*
196. A young man developed keratitis associated with the use of contact lenses that had been immersed in a contaminated cleaning solution. The most common bacterial etiologic agent in such cases is
 - A. *Chryseobacterium meningosepticum*
 - B. *Pseudomonas aeruginosa*
 - C. *Francisella tularensis*
 - D. *Escherichia coli*
197. The organism that is linked to peptic ulcers and is the most frequent cause of gastritis is
 - A. *Campylobacter jejuni*
 - B. *Helicobacter pylori*
 - C. *Salmonella* Typhimurium
 - D. *Yersinia enterocolitica*
198. New York City agar was developed for the isolation of
 - A. *Bordetella pertussis*
 - B. *Campylobacter fetus*
 - C. *Haemophilus influenzae*
 - D. *Neisseria gonorrhoeae*
199. Besides *Pseudomonas aeruginosa*, which of the following is an important cause of lower respiratory tract infections in patients with cystic fibrosis?
 - A. *Actinobacillus actinomycetem-comitans*
 - B. *Burkholderia cepacia*
 - C. *Chryseobacterium meningosepticum*
 - D. *Serratia marcescens*
200. *Eikenella corrodens* is an opportunistic pathogen, but it is most noted for causing
 - A. Abscesses of the oral cavity
 - B. Pneumonia
 - C. Postsurgical wound infections
 - D. Urinary tract infections

Mycobacteria

201. Mycobacteria have a large amount of a component in their cell wall that other bacteria lack. That component is
 - A. Lipids
 - B. Murein
 - C. Sterols
 - D. Teichoic acid
202. The species of *Mycobacterium* that would be most commonly associated with contamination of the hot water system in large institutions such as hospitals is
 - A. *M. haemophilum*
 - B. *M. marinum*
 - C. *M. ulcerans*
 - D. *M. xenopi*
203. *Mycobacterium fortuitum*, a rapidly growing *Mycobacterium*, grows on MacConkey agar in 5 days. Which other species of *Mycobacterium* is able to demonstrate growth within the same time period on MacConkey agar?
 - A. *M. bovis*
 - B. *M. chelonae*
 - C. *M. kansasii*
 - D. *M. tuberculosis*
204. A slowly growing, orange-pigmented, acid-fast bacillus was isolated from a cervical lymph node of a child with symptoms of cervical adenitis. The most likely etiologic agent in this case would be
 - A. *Mycobacterium avium-intracellulare* complex
 - B. *Mycobacterium chelonae*
 - C. *Mycobacterium fortuitum*
 - D. *Mycobacterium scrofulaceum*
205. When clinical specimens are processed for the recovery of *Mycobacterium tuberculosis*, the generally recommended method for digestion and decontamination of the sample is
 - A. 6% NaOH
 - B. HCl
 - C. NALC-NaOH
 - D. Trisodium phosphate
206. The etiologic agent of Hansen disease is
 - A. *Mycobacterium bovis*
 - B. *Mycobacterium fortuitum*
 - C. *Mycobacterium leprae*
 - D. *Mycobacterium tuberculosis*
207. The finding of five to six acid-fast bacilli per field ($\times 800$ to $\times 1000$) in a fuchsin smear of expectorated sputum should be reported as
 - A. 1+
 - B. 2+
 - C. 3+
 - D. 4+
208. Mycobacteria can be examined by using the
 - A. Dieterle stain
 - B. Gimenez stain
 - C. Kinyoun stain
 - D. Wright's stain
209. Which of the following is *not* characteristic of *Mycobacterium kansasii*?
 - A. Slow growing
 - B. Obligate aerobe
 - C. Nonchromogenic
 - D. Infections usually acquired by respiratory route
210. Which of the following mycobacteria has an optimal growth temperature 30–32°C?
 - A. *M. avium* complex
 - B. *M. bovis*
 - C. *M. ulcerans*
 - D. *M. xenopi*

211. Which one of the following drugs is *not* considered as primary antimycobacterial therapy?
 - A. Isoniazid
 - B. Kanamycin
 - C. Rifampin
 - D. Pyrazinamide
212. In the decontamination and digestion procedure for the isolation of mycobacteria from sputa samples, what is the role of NALC?
 - A. Bactericidal for normal flora
 - B. Liquefies mucus
 - C. Growth stimulant
 - D. Neutralizes pH to prevent damage to mycobacteria
213. Which one of the following tests would be appropriate in the diagnosis of a mycobacterial infection?
 - A. Elek test
 - B. CAMP test
 - C. Nagler test
 - D. PPD test
214. Which of the following is associated with livestock and causes a zoonosis?
 - A. *M. bovis*
 - B. *M. fortuitum*
 - C. *M. gordonae*
 - D. *M. xenopi*
215. Which of the following *Mycobacterium* is most noted for being associated with patients with acquired immunodeficiency syndrome?
 - A. *M. avium-intracellulae* complex
 - B. *M. marinum*
 - C. *M. kansasii*
 - D. *M. bovis*
216. Which of the following *Mycobacterium* produces an orange pigment and is most commonly recovered from water?
 - A. *M. intracellulare*
 - B. *M. gordonae*
 - C. *M. asiaticum*
 - D. *M. kansasii*
217. Which of the following *Mycobacterium* appears as buff-colored colonies after exposure to light and is niacin positive?
 - A. *M. bovis*
 - B. *M. scrofulaceum*
 - C. *M. tuberculosis*
 - D. *M. ulcerans*
218. The *Mycobacterium* that is the etiologic agent of “swimming pool granuloma” is
 - A. *M. fortuitum*
 - B. *M. kansasii*
 - C. *M. marinum*
 - D. *M. xenopi*
219. Susceptibility to thiophene-2-carboxylic acid hydrazide (T2H) is characteristic of which of the following mycobacteria?
 - A. *M. avium-intracellulae* complex
 - B. *M. bovis*
 - C. *M. kansasii*
 - D. *M. tuberculosis*
220. Which of the following is a fluorescent stain for mycobacteria?
 - A. Auramine-rhodamine
 - B. Calcofluor white
 - C. Fluorescein isothiocyanate
 - D. Ziehl-Neelsen
221. The most common photochromogenic *Mycobacterium* isolated in the U.S. is
 - A. *M. bovis*
 - B. *M. kansasii*
 - C. *M. tuberculosis*
 - D. *M. xenopi*

222. Skin cultures for the recovery of *Mycobacterium* spp. should be incubated at
 A. 22°C
 B. 30°C
 C. 35°C
 D. 42°C
223. Which of the following media would *not* be routinely used to culture *Mycobacterium* spp.?
 A. Lowenstein-Jensen-Gruft
 B. Lowenstein-Jensen
 C. Middlebrook 7H11
 D. Chocolate agar
224. Which of the following specimens is routinely decontaminated when trying to recover *Mycobacterium* spp.?
 A. Sputum
 B. Pleural fluid
 C. Lung biopsy
 D. Cerebrospinal fluid
225. Which of the following is *not* true of *Mycobacterium leprae*?
 A. Causes Hansen disease
 B. Difficult to grow *in vitro*
 C. Easily transmitted from person to person
 D. Usually grows in peripheral limbs of infected patients
228. An anaerobically incubated blood agar plate shows colonies surrounded by an inner zone of complete red cell lysis and an outer zone of incomplete cell lysis (double zone of hemolysis). The most likely presumptive identification of this isolate would be
 A. *Clostridium perfringens*
 B. *Clostridium tetani*
 C. *Fusobacterium nucleatum*
 D. *Prevotella melaninogenica*
229. A cervical mucosal abscess specimen was sent to the laboratory for bacteriologic examination. The culture of this sample grew an anaerobic gram-negative bacillus that was inhibited by bile, produced a black pigment, and was negative for indole production and positive for glucose, sucrose, and lactose fermentation. This isolate would most likely be
 A. *Bacteroides fragilis* group
 B. *Bacteroides ureolyticus*
 C. *Porphyromonas gingivalis*
 D. *Prevotella melaninogenica*
230. Which one of the following is *not* true of *Clostridium tetani*?
 A. It produces rapid tissue necrosis.
 B. It is a gram-positive, spore-forming bacillus.
 C. Microorganisms in soil contaminate puncture wounds.
 D. Disease is caused by an exotoxin acting on the central nervous system.
231. The characteristic colony morphology of *Actinomyces israelii* on solid agar resembles
 A. "Medusa head"
 B. A molar tooth
 C. A fried egg
 D. Ground glass

Anaerobic Bacteria

226. The potentially lethal intoxication type of food poisoning often associated with improperly canned food is caused by
 A. *Bacteroides fragilis*
 B. *Clostridium botulinum*
 C. *Clostridium perfringens*
 D. *Clostridium septicum*
227. Which of the following is *not* considered a zoonotic disease?
 A. Anthrax
 B. Botulism
 C. Brucellosis
 D. Leptospirosis

232. What is the predominant indigenous flora of the colon?
- Anaerobic, gram-negative, non-spore-forming bacteria
 - Anaerobic, gram-positive, non-spore-forming bacteria
 - Aerobic, gram-negative, non-spore-forming bacteria
 - Aerobic, gram-positive, spore-forming bacteria
233. Obligately anaerobic, gram-negative bacilli, recovered from an abdominal wound, were found to be resistant to penicillin. Growth of this organism was not inhibited by bile. What is the most likely identification of this isolate?
- Bacteroides fragilis* group
 - Clostridium septicum*
 - Eubacterium lentum*
 - Fusobacterium nucleatum*
234. Which of the following is described as obligately anaerobic gram-positive cocci?
- Capnocytophaga*
 - Peptostreptococcus*
 - Propionibacterium*
 - Veillonella*
235. Color Plate 29 ■ shows the filamentous gram-positive rod recovered from an aspirate of a closed chest abscess. It grew only under anaerobic conditions and was not acid-fast. What is the most likely presumptive identification of the isolate seen?
- Actinomyces israelii*
 - Bacteroides fragilis*
 - Clostridium septicum*
 - Propionibacterium acnes*
236. Growth on kanamycin-vancomycin laked blood agar incubated anaerobically is primarily used for
- Bacteroides fragilis*
 - Bifidobacterium dentium*
 - Clostridium perfringens*
 - Peptostreptococcus anaerobius*
237. The diagnosis of pseudomembranous colitis (*Clostridium difficile* associated disease) is often made by
- Serology
 - Culturing blood specimens
 - Assays to detect toxin in stool
 - Acid-fast stain of fecal material
238. The anaerobic, gram-negative, curved, motile bacilli associated with bacterial vaginosis belong to the genus
- Actinomyces*
 - Bifidobacterium*
 - Lactobacillus*
 - Mobiluncus*
239. An infant was seen in the emergency department with symptoms of neuromuscular weakness and constipation. The diagnosis of infant botulism was confirmed by the demonstration of toxin in the child's stool. The child most likely contracted this disease by
- A scratch wound caused by a cat
 - Ingestion of spores that germinated in the intestine
 - A puncture wound with a contaminated household item
 - Ingestion of preformed toxin found in a contaminated jar of pureed vegetables

240. The majority of the gram-positive, non-spore-forming, anaerobic bacilli isolated from clinical material will be
 A. *Bifidobacterium dentium*
 B. *Capnocytophaga ochracea*
 C. *Eubacterium limosum*
 D. *Propionibacterium acnes*
241. Which of the following clostridia has a terminal spore that causes the cell to swell?
 A. *C. botulinum*
 B. *C. difficile*
 C. *C. perfringens*
 D. *C. tetani*
242. The gram-negative, non-spore-forming, anaerobic bacillus frequently implicated in serious clinical infections such as brain and lung abscesses is
 A. *Bacteroides urealyticus*
 B. *Eubacterium lentum*
 C. *Fusobacterium nucleatum*
 D. *Peptostreptococcus anaerobius*
243. Which one of the following is a non-spore-forming, gram-positive, anaerobic bacillus?
 A. *Clostridium*
 B. *Fusobacterium*
 C. *Propionibacterium*
 D. *Veillonella*
244. Which of the following statements is *not* characteristic of *Clostridium botulinum*?
 A. Infant botulism is the most common clinical form.
 B. Pathogenicity is related to a potent neurotoxin.
 C. Oval spores are located terminally.
 D. Of the seven toxogenic types, types A, B, E, and F are associated with human botulism.
245. A tube of semisolid medium that contains resazurin appears pink. What does this indicate?
 A. Acid environment
 B. Alkaline environment
 C. Motility
 D. Presence of oxygen
246. Identify the *Fusobacterium* sp. considered to be the most frequent isolate recovered from clinical infections.
 A. *F. varium*
 B. *F. nucleatum*
 C. *F. mortiferum*
 D. *F. necrophorum*
247. Septicemia caused by which of the following is generally associated with an underlying malignancy?
 A. *Bifidobacterium dentium*
 B. *Clostridium septicum*
 C. *Eubacterium lentum*
 D. *Lactobacillus cateniforme*
248. Which of the following is the most potent bacterial exotoxin known?
 A. Botulinum toxin
 B. Erythrogenic toxin
 C. *C. difficile* toxin B
 D. *C. perfringens* alpha-toxin
249. Which *Clostridium* sp. is most commonly recovered from cases of gas gangrene?
 A. *C. bifermentans*
 B. *C. perfringens*
 C. *C. sordellii*
 D. *C. difficile*
250. Which of the following organisms is *not* gram-positive?
 A. *Eubacterium lentum*
 B. *Bifidobacterium dentium*
 C. *Propionibacterium acnes*
 D. *Suttonella wadsworthensis*

251. Which of the following statements is *not* true of clostridia?
- Pseudomembranous colitis is due to a toxin produced by *C. difficile*.
 - Clinically significant clostridia are found in the normal flora of the colon and in the soil.
 - Botulism is caused by ingesting preformed toxin and can be prevented by boiling food prior to eating.
 - C. tetani* spores will form in the presence of oxygen; therefore, anaerobiosis in a wound is not required to cause tetanus.
252. Gram-positive bacilli with central spores are seen in the direct Gram stain of a tissue biopsy. After 24 hours of incubation, no growth is seen on the sheep blood agar plate incubated aerobically and the chocolate agar plate incubated in increased CO₂. Which of the following is the likely cause of the infection?
- Bacillus*
 - Clostridium*
 - Lactobacillus*
 - Prevotella*
253. Small alpha-hemolytic colonies are seen on a sheep blood agar plate after 48 hours of incubation on a vaginal culture. This describes which of the following?
- Bacteroides*
 - Clostridium*
 - Lactobacillus*
 - Porphyromonas*
254. Which of the following statements is true regarding *Clostridium perfringens*?
- There are five serologic types.
 - Spores are terminally located.
 - Alpha-toxin is produced by all strains.
 - Spores are readily seen in laboratory media.
255. Which is a correct statement regarding *Clostridium tetani*?
- It is proteolytic
 - It is lecithinase positive
 - It is characteristically nonmotile
 - It produces terminal spores
256. Which anaerobic, gram-negative rod can be presumptively identified by its Gram stain morphology, and inhibition by bile and a 1-μg kanamycin disk?
- Bacteroides fragilis* group
 - Eubacterium lentum*
 - Fusobacterium nucleatum*
 - Porphyromonas gingivalis*
257. Which of the following tests is most appropriate for the presumptive identification of *Prevotella melaninogenica*?
- SPS sensitivity test
 - Nagler test
 - Cytotoxin assay
 - Fluorescence test
258. Which of the following tests is most appropriate for the identification of *Clostridium difficile*?
- SPS sensitivity test
 - Nagler test
 - Cytotoxin assay
 - Fluorescence test
259. Which of the following tests is most appropriate for the presumptive identification of *Clostridium perfringens*?
- SPS sensitivity test
 - Reverse CAMP test
 - Cytotoxin assay
 - Esculin hydrolysis

260. Which of the following tests is most appropriate for the presumptive identification of *Peptostreptococcus anaerobius*?
- SPS disk
 - Colistin disk
 - Kanamycin disk
 - Vancomycin disk
261. A curved appearance on Gram stain is characteristic of which of the following?
- Actinomyces israelii*
 - Clostridium septicum*
 - Fusobacterium nucleatum*
 - Propionibacterium acnes*
262. Purulent material from a cerebral abscess was submitted to the laboratory for smear and culture. On direct Gram stain, gram-positive cocci in chains and gram-negative bacilli with pointed ends were seen. Plates incubated aerobically exhibited no growth at 24 hours. On the basis of the organisms seen on the smear, what is the most likely presumptive identification of the etiologic anaerobic agents?
- Veillonella* sp. and *Clostridium* sp.
 - Eubacterium* sp. and *Veillonella* sp.
 - Peptostreptococcus* sp. and *Nocardia* sp.
 - Fusobacterium* sp. and *Peptostreptococcus* sp.
263. Which of the following is an important virulence factor of *Bacteroides fragilis*?
- Endotoxin
 - Exotoxins
 - Polysaccharide capsule
 - Protease
264. Which of the following is *not* true of *Bacteroides fragilis*?
- Lipase and lecithinase negative
 - Anaerobic gram-negative bacillus
 - Commonly associated with intra-abdominal infections
 - Among the most antimicrobial-sensitive anaerobic bacteria
265. Aspiration of vomitus can lead to pneumonia. Which of the following would *not* be a likely causative agent in aspiration pneumonia?
- Bacteroides gracilis*
 - Mobiluncus* sp.
 - Porphyromonas* sp.
 - Prevotella melaninogenica*
266. To ensure that anaerobic conditions have been achieved in anaerobic jars or chambers, an oxygen-sensitive indicator is employed, such as
- Bromcreosol purple
 - Methylene blue
 - Methyl red
 - Phenol red
267. Egg yolk agar showing a precipitate in the medium surrounding the colony is positive for
- Lecithinase production
 - Lipase production
 - Protease activity
 - Starch hydrolysis
268. After 72 hours of anaerobic incubation, small olive-green to black colonies are seen. A Gram stain reveals gram-positive cocci. What is the most likely identification of this organism?
- Fingoldia magna*
 - Peptococcus niger*
 - Peptostreptococcus anaerobius*
 - Veillonella parvula*
269. Which of the following is an important cause of food poisoning?
- Bacteroides fragilis*
 - Bacteroides ureolyticus*
 - Clostridium perfringens*
 - Clostridium histolyticum*

270. Which bacterium is part of the normal vaginal flora that helps resist the onset of bacterial vaginosis?
- Peptostreptococcus* sp.
 - Peptococcus* sp.
 - Lactobacillus* sp.
 - Mobiluncus* sp.

Chlamydia*, *Rickettsia*, and *Mycoplasma

271. *Chlamydia trachomatis* causes which of the following?
- Rat-bite fever
 - Inclusion conjunctivitis
 - A skin disease found predominantly in tropical areas
 - Zoonosis in birds and parrot fever in humans
272. Which one of the following microorganisms *cannot* be cultivated on artificial cell-free media?
- Chlamydia trachomatis*
 - Mycoplasma hominis*
 - Mycoplasma pneumoniae*
 - Ureaplasma urealyticum*
273. The etiologic agent of primary atypical pneumonia is
- Chlamydia trachomatis*
 - Chlamydia psittaci*
 - Mycoplasma pneumoniae*
 - Ureaplasma urealyticum*
274. The recommended medium for the recovery of *Mycoplasma pneumoniae* from clinical specimens is
- Charcoal yeast extract medium
 - Fletcher semisolid medium
 - Middlebrook
 - SP4 agar
275. *Chlamydophila (Chlamydia) psittaci* infections in humans most commonly result after exposure to infected
- Amphibians
 - Arthropods
 - Avians
 - Mammals
276. Which of the following is *not* true of *Coxiella burnetii*?
- It is an obligate intracellular parasite.
 - It is transmitted from animals to humans by inhalation.
 - A rash appears first on the extremities and then on the trunk.
 - Is the etiologic agent of Q fever, which may be acute or chronic.
277. Which of the following is *true* about mycoplasmas?
- Resistant to penicillin
 - Not able to survive extracellularly
 - Easily stained using the Gram stain
 - Grow on routine nonselective culture media
278. Corneal scrapings are collected and examined microscopically using a direct fluorescent test to detect inclusion bodies for the diagnosis of infection caused by
- Chlamydia trachomatis*
 - Ehrlichia chaffeensis*
 - Mycoplasma hominis*
 - Rickettsia prowazekii*
279. Which of the following *Mycoplasmataceae* has *not* been connected with human genital infections?
- Mycoplasma genitalium*
 - Mycoplasma hominis*
 - Mycoplasma pneumoniae*
 - Ureaplasma urealyticum*

280. Which of the following is *not* true about *Chlamydophila* (*Chlamydia*) *pneumoniae*?
- Common agent of lower respiratory tract infection
 - Humans become infected from animal reservoirs.
 - Tetracycline and erythromycin are effective treatments.
 - Research has found an association with atherosclerosis.
281. What is the reference method for detection of *Chlamydia trachomatis* in cases of sexually transmitted disease?
- Tissue culture
 - Nonculture EIA methods
 - DNA-amplification techniques
 - Culture on modified Thayer-Martin agar
282. Colonies said to have the appearance of a “fried egg” are characteristic of
- Ehrlichia chaffeensis*
 - Mycoplasma genitalium*
 - Mycoplasma hominis*
 - Ureaplasma urealyticum*
283. Human infection with the causative agent of Q fever is acquired by
- Inhalation of infectious material
 - The bite of a mite (chigger)
 - The bite of a body louse
 - The bite of the arthropod *Phlebotomus*
284. For nonspecific staining of *Rickettsia* the recommended stain is
- Gimenez stain
 - Gomori silver stain
 - Gram stain
 - Kinyoun stain
285. Rocky Mountain spotted fever is transmitted by the bite of a tick infected with
- Rickettsia akari*
 - Rickettsia conorii*
 - Rickettsia prowazekii*
 - Rickettsia rickettsii*
286. Transmission of the sylvatic form of typhus infection caused by *Rickettsia prowazekii* is associated with
- Bats
 - Rabbits
 - Raccoons
 - Squirrels
287. The mild type of typhus fever that is caused by recrudescence of an initial attack of epidemic typhus is known as
- Brill-Zinsser disease
 - Q fever
 - São Paulo typhus
 - Tsutsugamushi disease
288. The causative agent of endemic or murine typhus is
- Rickettsia akari*
 - Rickettsia conorii*
 - Rickettsia prowazekii*
 - Rickettsia typhi*
289. A screening test for the identification of *Mycoplasma pneumoniae* isolates is the
- Acid-fast stain
 - Gram stain
 - Lysis of red blood cells
 - Catalase test
290. A genital specimen is inoculated into 10 B broth. After overnight incubation, an alkaline reaction is noted without turbidity. What is the most likely explanation?
- pH change due to molecules in the clinical specimen
 - Presence of *Mycoplasma genitalium*
 - Presence of *Mycoplasma hominis*
 - Presence of *Ureaplasma urealyticum*

Spirochetes

291. Detection of antibody against cardiolipin is useful for the diagnosis of which of the following diseases?
- Leptospirosis
 - Lyme disease
 - Relapsing fever
 - Syphilis
292. During the first week of leptospirosis, the most reliable way to detect the presence of the causative agent is by the direct
- Culturing of blood
 - Culturing of urine
 - Examination of blood
 - Examination of cerebrospinal fluid
293. Serious congenital infections are associated with
- Borrelia burgdorferi*
 - Borrelia recurrentis*
 - Treponema pallidum* subsp. *pallidum*
 - Treponema pallidum* subsp. *pertenue*
294. A helicoidal, flexible organism was demonstrated in a blood smear. This motile organism was approximately 12 μm long, approximately 0.1 μm wide, and had semicircular hooked ends. The description of this organism corresponds most closely to the morphology of
- Borrelia*
 - Leptonema*
 - Leptospira*
 - Treponema*
295. The etiologic agent of epidemic relapsing fever is *Borrelia recurrentis*, which is commonly transmitted by
- Fleas
 - Lice
 - Mosquitoes
 - Ticks
296. Which of the following is *not* true of the VDRL test?
- False-positive tests are more frequent than with the FTA-ABS test.
 - The antibody titer will decline if the patient is adequately treated.
 - Inactivated *Treponema pallidum* serves as the antigen.
 - The test is usually positive in secondary syphilis.
297. *Borrelia burgdorferi*, a spirochete transmitted by *Ixodes dammini* in the northeastern U.S., is the etiologic agent of
- Lyme disease
 - Rat-bite fever
 - Relapsing fever
 - Q fever
298. The axial fibrils of spirochetes most closely resemble which bacterial structure?
- Cytoplasmic membrane
 - Flagellum
 - Pilus
 - Sporangium
299. Which of the following is *not* correct regarding spirochetes?
- Motility is via axial filaments.
 - Spirochetes are gram positive.
 - They are visualized best using dark field or phase optics.
 - Those associated with human disease are 0.1–0.5 μm in diameter and 5–30 μm in length

300. A positive VDRL test for syphilis was reported on a young woman known to have hepatitis. When questioned by her physician, she denied sexual contact with any partner symptomatic for a sexually transmitted disease. Which of the following would be the appropriate next step for her physician?
- Treat her with penicillin
 - Identify her sexual contacts for serologic testing
 - Test her serum using a fluorescent treponemal antibody-absorbed assay
 - Reassure her that it was a biologic false-positive caused by her liver disease

Antimicrobial Agents and Antimicrobial Susceptibility Testing

301. A suspension of the test organism for use in broth dilution and disk diffusion testing is adjusted to match the turbidity of a
- #0.5 McFarland standard
 - #1.0 McFarland standard
 - #2.0 McFarland standard
 - #3.0 McFarland standard
302. When testing the antimicrobial susceptibility of *Haemophilus influenzae* strains by disk-agar diffusion, the recommended medium is
- Chocolate agar
 - Charcoal yeast extract agar
 - Mueller-Hinton base supplemented with 5% sheep blood
 - Mueller-Hinton base supplemented with hematin, NAD, and yeast extract
303. The chemotherapeutic agents structurally similar to the vitamin *p*-aminobenzoic acid that act by inhibiting bacteria via inhibition of folic acid synthesis are
- Aminoglycosides
 - Penicillins
 - Macrolides
 - Sulfonamides
304. Which of the following organisms would *not* routinely undergo antimicrobial susceptibility testing?
- Escherichia coli*
 - Proteus mirabilis*
 - Staphylococcus aureus*
 - Streptococcus pyogenes*
305. Penicillin is active against bacteria by
- Inhibition of protein synthesis at the 30S ribosomal subunit
 - Reduction of dihydrofolic acid
 - Inhibition of peptidoglycan synthesis
 - Inhibition of nucleic acid function
306. The minimum bactericidal concentration (MBC) of an antimicrobial agent is defined as the lowest concentration of that antimicrobial agent that kills at least _____ of the original inoculum.
- 95.5%
 - 97%
 - 99.9%
 - 100%
307. Resistance to clindamycin can be induced *in vitro* by
- Ampicillin
 - Erythromycin
 - Gentamicin
 - Penicillin

308. The term that denotes a situation in which the effect of two antimicrobial agents together is greater than the sum of the effects of either drug alone is
- A. Additivism
 - B. Antagonism
 - C. Sensitivity
 - D. Synergism
309. Beta-lactamase-producing strains of *Haemophilus influenzae* are resistant to
- A. Chloramphenicol
 - B. Erythromycin
 - C. Penicillin
 - D. Trimethoprim sulfamethoxazole
310. The agar recommended by the Clinical and Laboratory Standards Institute for routine susceptibility testing of nonfastidious bacteria is
- A. MacConkey agar
 - B. Middlebrook 7H10 agar
 - C. Mueller-Hinton agar
 - D. Trypticase soy agar
311. The pH of the agar used for the Kirby-Bauer test should be
- A. 7.0–7.2
 - B. 7.2–7.4
 - C. 7.4–7.6
 - D. 7.6–7.8
312. Which drug known to be active against parasitic infections has importance as a therapeutic agent in cases of disease caused by anaerobic bacteria?
- A. Isoniazid
 - B. Metronidazole
 - C. Rifampin
 - D. Trimethoprim
313. An example of a bactericidal antibiotic is
- A. Chloramphenicol
 - B. Erythromycin
 - C. Tetracycline
 - D. Tobramycin
314. The extended-spectrum beta-lactamases confer resistance to
- A. Amoxicillin
 - B. Ceftriaxone
 - C. Erythromycin
 - D. Rifampin
315. Which of the following media should be used for *in vitro* susceptibility testing of *S. pneumoniae*?
- A. Chocolate agar
 - B. Charcoal yeast extract agar
 - C. Mueller-Hinton base supplemented with 5% lysed horse blood
 - D. Mueller-Hinton base supplemented with 1% hemoglobin and 1% IsoVitaleX
316. Rapid testing for beta-lactamase production is recommended, before initiation of antimicrobial therapy, for isolates of
- A. *Serratia marcescens*
 - B. *Haemophilus influenzae*
 - C. *Staphylococcus epidermidis*
 - D. *Streptococcus pyogenes*
317. The phenomenon of bacterial resistance to the bactericidal activity of penicillins and cephalosporins, with only inhibition of the organism's growth, is known as
- A. High-level resistance
 - B. Intrinsic resistance
 - C. Inducible resistance
 - D. Tolerance

318. The supervisor of a microbiology laboratory has been asked to begin performing *in vitro* antimicrobial susceptibility testing of *Mycobacterium tuberculosis* because of an increase in the reported resistance in his community. Which of the following methods would be appropriate for this testing?
- A. Broth microdilution method using Mueller-Hinton broth
 - B. Kirby-Bauer method
 - C. BACTEC method
 - D. Schlichter method
319. Clavulanic acid is classified as a
- A. Beta-lactam
 - B. Beta-lactamase inhibitor
 - C. Macrolide
 - D. Aminoglycoside
320. Which of the following antimicrobial agents acts by inhibiting cell wall synthesis?
- A. Clindamycin
 - B. Gentamicin
 - C. Naladixic acid
 - D. Vancomycin
321. Metronidazole is most commonly recommended for treatment of infections caused by
- A. Aerobic microorganisms
 - B. Microaerophilic microorganisms
 - C. Obligate anaerobic microorganisms
 - D. Obligate intracellular microorganisms
322. Which of the following antimicrobial agents acts by inhibiting protein synthesis?
- A. Gentamicin
 - B. Methicillin
 - C. Rifampin
 - D. Ampicillin
323. Chloramphenicol is an important antimicrobial agent for the treatment of meningitis as well as several other serious infections. Unfortunately, chloramphenicol exhibits significant complications that limit its clinical usefulness. These effects include
- A. Allergic reactions and anaphylaxis
 - B. Bone marrow suppression and aplastic anemia
 - C. Significant gastrointestinal manifestations
 - D. Photosensitivity
324. Which of the following is *not* one of the standard control organisms used for the weekly testing of antimicrobial disks?
- A. *Staphylococcus epidermidis* (ATCC 25833)
 - B. *Pseudomonas aeruginosa* (ATCC 27853)
 - C. *Escherichia coli* (ATCC 25922)
 - D. *Enterococcus faecalis* (ATCC 29212)
325. When using the rapid chromogenic cephalosporin method for the detection of beta-lactamase production by an organism, a positive test is indicated by the color
- A. Yellow
 - B. Green
 - C. Red
 - D. Blue
- Procedures and Biochemical Identification of Bacteria**
326. Which of the following body sites is *not* normally colonized by large numbers of normal flora organisms?
- A. Colon
 - B. Skin
 - C. Lungs
 - D. Vagina

327. During childbearing years, the normal flora of the vagina is predominantly
A. *Enterococcus*
B. *Lactobacillus*
C. *Propionibacterium*
D. Coagulase-negative *Staphylococcus*
328. The MIDI identification system is based on
A. Antibigrams
B. Colony pigment on ChromAgar
C. Fatty acid analysis
D. Multiple biochemical tests
329. Which of the following is *not* a correct statement regarding blood cultures?
A. Collection of 10–20 mL per culture for adults is recommended
B. Two or three blood cultures are recommended as optimum
C. Volume of blood cultured is more critical than timing of culture
D. Blood drawn for culture may be allowed to clot
330. In capnophilic incubators, carbon dioxide concentrations should be maintained between
A. 1% and 5%
B. 5% and 10%
C. 10% and 15%
D. 15% and 20%
331. The recommended anticoagulant for use when a body fluid or joint fluid that may clot is sent for microbiologic examination is
A. Heparin
B. Sodium polyethanolsulfonate (SPS)
C. Sodium EDTA
D. Sodium citrate
332. Suggested quality control organisms for Simmons citrate agar slants are
A. *Shigella sonnei*; *Escherichia coli*
B. *Klebsiella pneumoniae*; *Escherichia coli*
C. *Escherichia coli*; *Edwardsiella tarda*
D. *Morganella morganii*; *Edwardsiella tarda*
333. Laboratory professionals are at risk for disease transmission. The majority of cases of laboratory-related infections are associated with
A. Infectious aerosols
B. Contamination of abraded skin
C. Puncture wounds
D. Person-to-person transmission
334. The ability of a microorganism to deaminate phenylalanine can be assessed by inoculating a phenylalanine agar slant with the test organism. Following incubation, if the organism is positive, a green color develops with the addition of
A. 10% ferric chloride
B. 2% sulfanilamide
C. 2 N sodium carbonate
D. 5% alpha-naphthol
335. Which of the following media is both selective and differential?
A. Sheep blood agar
B. Chocolate agar
C. Mannitol salt agar
D. Mueller-Hinton agar
336. The paper strip test for the demonstration of hydrogen sulfide production is impregnated with a solution of
A. Sodium desoxycholate
B. Lead acetate
C. Potassium tellurite
D. Sodium thiosulfate

337. In the nitrate test, reduction is demonstrated by the development of a red color, following the addition of
- Alpha-naphthol and potassium hydroxide
 - p*-Dimethylaminobenzaldehyde and amyl alcohol
 - Ninhydrin and acetone
 - Alpha-naphthylamine and sulfanilic acid
338. The Moeller test for the detection of decarboxylase activity is dependent upon
- An alkaline pH shift in the medium
 - The oxidation of gluconate
 - An acid pH basal medium
 - A deamination of tryptophan
339. The medium used to determine whether an organism is oxidative or fermentative with respect to its metabolic activities is
- CTA medium
 - OF medium
 - HE medium
 - XLD medium
340. Methyl red-Voges Proskauer (MRVP) broth is inoculated and incubated for 48 hours. What two reagents must be added to determine if the bacterium is VP positive?
- Creatine and 1 N HCl
 - 10% FeCl₃ and alpha-naphthol
 - Kovac's reagent and zinc dust
 - Alpha-naphthol and 40% KOH
341. Which of the following is *not* true of blood cultures for the recovery of bacteria?
- No more than three cultures should be drawn in 1 day.
 - Cultures should be drawn before the expected fever spike.
 - Cultures should be incubated aerobically and anaerobically.
 - Collect 5 mL of blood for optimal recovery of pathogen.
342. Continuous blood culture systems that detect changes in headspace pressure in bottles are referred to as
- Fluorescence methods
 - ¹⁴C detection methods
 - Colorimetry methods
 - Manometric methods
343. Which of the following statements does *not* apply to the acridine orange stain?
- Binds to the teichoic acid of the cell wall
 - Requires the use of a fluorescence microscope
 - Is more sensitive than the Gram stain
 - Is recommended for fluid and exudates with low bacterial concentrations
344. In the catalase test, the formation of bubbles is due to
- Production of oxygen (O₂)
 - Production of hydrogen gas (H₂)
 - Breakdown of water
 - Oxidation of hydrogen peroxide
345. Which of the following would *not* be appropriate for a positive control in the PYR test?
- Staphylococcus aureus*
 - Staphylococcus lugdunensis*
 - Streptococcus pyogenes*
 - Enterococcus faecalis*

Case Studies

346. A purulent aspirate of joint fluid from a 28-year-old female with joint pain was sent for microbiologic examination. The Gram stain of this sample revealed many polymorphonuclear cells with intracellular and extracellular gram-negative diplococci. Given the specimen type and microscopic findings, the appropriate selective medium for primary isolation would be
- Mannitol salt agar
 - Potassium tellurite agar
 - Modified Thayer-Martin agar
 - Cefsulodin-irgasan-novobiocin
347. A 21-year-old sexually active woman came to the university student health service with a 2-day history of urinary frequency with urgency, dysuria, and hematuria. She had no history of prior urinary tract infection. Laboratory test showed a white blood cell count of $10 \times 10^9/\text{L}$. The urine sediment contained innumerable white cells. Cultures yielded more than 10^5 colony-forming units/mL of a lactose-fermenting gram-negative rod. The most likely etiologic agent in this case is
- Escherichia coli*
 - Klebsiella pneumoniae*
 - Morganella morganii*
 - Proteus mirabilis*
348. A 36-year-old man was seen in the emergency department. He complained of fever and headache. He had returned 1 week previously from a 6-week visit to a village in India. Among the differential diagnoses was typhoid fever. What is the most critical laboratory test necessary to establish or eliminate the diagnosis?
- Blood cultures
 - Sputum cultures
 - Stool cultures
 - Urine cultures
349. A 45-year-old man was seen in the emergency department with fever, chills, nausea, and myalgia. He reported that 2 days earlier he had eaten raw oysters at a popular seafood restaurant. On admission he was febrile and had hemorrhagic, fluid-filled bullous lesions on his left leg. The patient had a history of diabetes mellitus, chronic hepatitis B, and heavy alcohol consumption. The patient, who had a temperature of 102.2°F , was admitted to the intensive care unit for presumed sepsis, and treatment was begun. A curved gram-negative rod was isolated from blood cultures drawn on admission and fluid from the bullous leg wound. On the third day, disseminated intravascular coagulation developed, and he died. The source of the oysters eaten by the deceased patient was the Gulf of Mexico. The most likely etiologic agent in this case would be
- Aeromonas hydrophila*
 - Plesiomonas shigelloides*
 - Vibrio vulnificus*
 - Yersinia enterocolitica*

350. A 3-year-old was brought to the emergency department by her parents. She had been febrile with a loss of appetite for the past 24 hours. Most recently the parents noted that it was difficult to arouse her. She attended a daycare center, and her childhood immunizations were current. On examination she demonstrated a positive Brudzinski sign indicative of meningeal irritation. Cultures of blood and cerebrospinal fluid (CSF) were sent to the laboratory. Her CSF was cloudy, and the Gram stain showed many polymorphonuclear cells containing gram-negative diplococci. The white blood cell count was $25 \times 10^9/\text{L}$, with 88% polymorphonuclear cells. The CSF protein was 100 mg/dL, and the glucose was 15 mg/dL. Cultures of the blood and CSF grew the same organism. The most likely etiologic agent in this case is
- Haemophilus influenzae*
 - Listeria monocytogenes*
 - Moraxella catarrhalis*
 - Neisseria meningitidis*
351. Gram-positive rods were recovered from the chest fluid drawn from a teenager with right lower lobe pneumonia who lived on a dairy farm. At 24 hours, pinpoint colonies grew on sheep blood agar that showed faint zones of beta-hemolysis. The isolate was catalase negative and demonstrated a positive CAMP test. Which of the following is the most likely etiologic agent in this case?
- Listeria monocytogenes*
 - Streptococcus agalactiae*
 - Arcanobacterium pyogenes*
 - Streptobacillus moniliformis*
352. A young woman complaining of symptoms of sudden onset of fever, vomiting, diarrhea, and rash was seen by her gynecologist. She was admitted to the hospital, where a culture of vaginal discharge grew many coagulase-positive staphylococci. The most likely diagnosis in this case would be
- Kawasaki disease
 - Pelvic inflammatory disease
 - Scalded skin syndrome
 - Toxic shock syndrome
353. A 32-year-old male was seen in the emergency department with symptoms of lower right quadrant abdominal pain and diarrhea. A complete blood count showed a leukocytosis with an increased number of neutrophils. He was admitted, and a stool culture was obtained. The culture showed many gram-negative bacilli, which were oxidase negative, citrate negative, and indole negative. The triple sugar iron reaction was acid over acid, but there was no evidence of gas or H_2S production. The organism was positive for urease and ONPG and negative for phenylalanine. The characteristic symptomatology and the biochemical reactions confirmed that the etiologic agent was
- Salmonella* Paratyphi
 - Shigella dysenteriae*
 - Vibrio parahaemolyticus*
 - Yersinia enterocolitica*

354. In August, a patient presented at a community hospital in New England with symptoms of a skin rash, headache, stiff neck, muscle aches, and swollen lymph nodes. A silver-stained biopsy of a skin lesion showed spirochetes. On the basis of the clinical syndrome and laboratory detection of a causative agent, the patient was diagnosed as having
- Lyme disease
 - Plague
 - Rabbit fever
 - Relapsing fever
355. Several international participants in an Eco-Challenge adventure race in Borneo became ill with symptoms of chills, diarrhea, headaches, and eye infections. The racers hiked in the mountains and jungles, swam in rivers, and slogged through flooded streams for 2 weeks. Contact with contaminated water and soil during the race was highly associated with illness. What is the most likely etiologic agent in this case?
- Borellia recurrentis*
 - Brucella canis*
 - Franciscella tularensis*
 - Leptospira interrogans*
356. An anemic patient was transfused with packed red blood cells. Approximately 1 hour after the transfusion began, the patient developed fever and hypotension consistent with endotoxic shock. The red blood cells had been stored at 4°C for approximately 30 days before their use. The organism most likely to be involved in this case is
- Campylobacter fetus*
 - Neisseria meningitidis*
 - Pseudomonas aeruginosa*
 - Yersinia enterocolitica*
357. A college student got a summer job working at a marina. While repairing the outboard motor on a rental boat, he received several lacerations on his right forearm. No medical treatment was sought at the time of the injury, but after several weeks he noted that the lesions were not healing and he sought the opinion of his physician. A biopsy of one of the lesions revealed a cutaneous granulomatous condition. Given the history, which of the following microorganisms would most likely be the etiologic agent in this case?
- Mycobacterium marinum*
 - Nocardia asteroides*
 - Pseudomonas aeruginosa*
 - Vibrio vulnificus*
358. A woman, who had recently returned from a vacation in Mexico, was admitted to the hospital. She was febrile and complained of flulike symptoms. Her case history revealed that she had eaten cheese that had been made from unpasteurized milk while on vacation. The most likely etiologic agent in this case would be
- Bordetella pertussis*
 - Listeria monocytogenes*
 - Staphylococcus aureus*
 - Yersinia enterocolitica*
359. A 7-year-old female became ill with an intestinal illness after visiting a petting zoo featuring farm animals such as calves, lambs, and chickens. She had bloody diarrhea and went on to develop hemolytic uremic syndrome. The most likely etiologic agent in this case is
- Escherichia coli* 0157:H7
 - Shigella dysenteriae*
 - Vibrio cholerae* 01
 - Vibrio cholerae* non01

360. A middle-aged man with a history of smoking and drinking for over 40 years developed shortness of breath, fever, frontal headache, diarrhea, and cough. He worked in the produce section of a supermarket, which routinely misted the fresh greens. His medical history included a kidney transplant several years ago for which he remains on antirejection therapy. His sputum Gram stain showed numerous polymorphonuclear cells but rare microorganisms. An X-ray of his chest showed an infiltrate in the left lower lobe, and a diagnosis of atypical pneumonia was made. Which of the following is the most likely etiologic agent in this case?
- A. *Bordetella pertussis*
 - B. *Klebsiella pneumoniae*
 - C. *Legionella pneumophila*
 - D. *Moraxella catarrhalis*



Aerobic Gram-Positive Bacteria

1.

C. *Enterococcus* and other group D streptococci can be presumptively identified based on their ability to hydrolyze esculin in the presence of 1–4% bile salts. The medium is made selective for enterococci by the addition of either sodium azide or 4% bile salts. Organisms able to grow on this medium and hydrolyze esculin produce esculetin, which reacts with an iron salt to form a black color in the agar.

2.

D. Strains of *Corynebacterium diphtheriae* infected by a lysogenic bacteriophage produce an extremely potent exotoxin. Absorption of the toxin may cause a rapidly fatal hypertoxic disease characterized by myocarditis and neuritis. This disease most commonly affects children aged 1 to 10 years. Transmission is by contact with a human carrier or with contaminated fomites.

3.

B. Staphylococcal enterocolitis food poisoning cases result from the ingestion of contaminated foods containing preformed thermostable enterotoxin. This form of intoxication causes a profuse and watery diarrhea due to the loss of electrolytes

and fluids into the lumen. In many cases, the causative agent may never be recovered from patient specimens.

4.

B. Nutritionally variant streptococci (NVS) are now termed *Abiotrophia*. These clinically significant microorganisms, which account for 5–6% of the cases of endocarditis, are frequently not able to be recovered because of insufficient quantities of vitamin B₆ in the culture medium. The routine use of a pyridoxal disk, a streak of *Staphylococcus*, or vitamin B₆-supplemented culture media is required for isolation.

5.

C. Scalded skin syndrome is a form of dermatitis produced by strains of *Staphylococcus aureus* that elaborate exfoliative toxin. Two types of this toxin have been identified: exfoliation A and exfoliation B. This potent toxin acts by disturbing the adhesive forces between cells of the stratum granulosum, which causes the appearance of the clear, large, flaccid bullae and the skin to peel off. Infants and children are most commonly affected with this form of dermatitis, beginning about the face and trunk and subsequently spreading to the extremities.

6.

A. Presumptive identification of group A streptococci can be achieved through the PYR (L-pyrrolidonyl- β -naphthylamide) disk test. The use of a 0.04-unit bacitracin disk is no longer recommended because groups C and G streptococci are also susceptible to this agent. A positive test result is interpreted as a bright red color change within 5 minutes.

7.

A. Staphylococci and micrococci are both catalase positive gram-positive cocci. Staphylococci are more clinically significant, so it is important to differentiate *Micrococcus* from *Staphylococcus*. Micrococci are modified oxidase positive, whereas staphylococci are negative.

8.

B. Nocardiosis is characterized by mycetoma or chronic suppurative infection. Draining sinus tracts in the subcutaneous tissue are a common manifestation of the disease. *Nocardia* spp. are soil saprophytes that may produce disease in humans either by the inhalation of contaminated material or through skin abrasions. Microscopic examination of pus from suspected cases will demonstrate partially acid-fast, gram-positive, branching filamentous or coccoid organism.

9.

A. Infection caused by *Erysipelothrix rhusiopathiae* in humans is primarily erysipeloid. Erysipeloid is usually the result of contact with an infected animal or contaminated animal product. The characteristic presentation is cutaneous spreading lesions of the fingers or hand that are raised and erythematous. Although generally confined to the skin, *E. rhusiopathiae* has been implicated in rare cases of endocarditis.

10.

A. *Streptococcus agalactiae* isolates can be presumptively identified by the demonstration of a positive CAMP reaction. CAMP is an acronym for the scientists (Christie, Atkins, Munch-Petersen) who discovered this phenomenon. Group B streptococci elaborate the CAMP factor, which acts to enhance the zone of hemolysis produced by beta-lysin-producing strains of *Staphylococcus aureus*. Incubation of test plates should be carried out in ambient air, because increased CO₂ and anaerobic incubation increase the rate of false-positive CAMP reactions by group A streptococci.

11.

D. The recovery rate of coagulase-negative *Staphylococcus saprophyticus* from urinary tract infections in young females is second only to that of *Escherichia coli*. The organism has a predilection for the epithelial cells of the urogenital tract and is often seen in large numbers adhering to these cells on Gram stain. Key to the identification of this coagulase-negative *Staphylococcus* is its resistance to novobiocin.

12.

A. The organism seen in Color Plate 26■ is *Listeria monocytogenes*. *Listeria* is an important animal and human pathogen that is known to cause abortion, meningitis, and septicemia in humans. This gram-positive rod is actively motile at room temperature (but not at 35°C), hydrolyzes esculin, produces catalase, and is oxidase negative. When recovered on sheep blood agar plates from clinical samples, it is often initially confused with group A or group B streptococci because of its beta-hemolysis.

13.

A. *Streptococcus agalactiae* (group B *Streptococcus*) is a principal cause of bacterial meningitis and septicemia in neonates. The organism, which is a part of the indigenous microbial flora of the vagina, is transmitted by the mother before birth, usually as the baby passes through the birth canal. Neonatal infection with group B streptococci may occur either as an early-onset disease (at birth) or as a delayed-onset syndrome that manifests itself weeks after birth.

14.

B. The vegetative cells and spores of *Bacillus cereus* are widely distributed in the environment. The virulence mechanisms of *B. cereus* are an enterotoxin and a pyogenic toxin. Accidents in nature resulting in cuts or abrasions contaminated with soil or vegetation, intravenous drug abuse, ingestion of contaminated foods, and traumatic introduction into a normally sterile site through the use of contaminated medical equipment are associated with infection.

15.

B. The formation of the characteristic *Corynebacterium diphtheriae* granules and cellular morphology seen in methylene blue stains is enhanced when the organism is grown on Loeffler's serum medium. Although this medium is primarily designed for the recovery of *C. diphtheriae* from clinical samples, it is not a differential medium. The agar slant, when inoculated, may demonstrate growth of corynebacteria within 8 to 24 hours.

16.

C. Tinsdale medium, for the primary isolation of *Corynebacterium diphtheriae*, not only inhibits indigenous respiratory flora but differentiates colonies of *C. diphtheriae*. The potassium tellurite in the medium is taken up by colonies of *Corynebacterium*, causing them to appear black.

Colonies of *C. diphtheriae* are presumptively identified when black colonies surrounded by a brown halo are seen on this agar medium. However, other corynebacteria and some staphylococci will produce a similar reaction.

17.

B. The Elek immunodiffusion test is recommended for detecting toxigenic strains of *Corynebacterium diphtheriae*. In the test, diphtheria antitoxin is impregnated on a sterile filter paper strip, which is pressed onto the surface of an Elek agar plate. Test and control strains are then inoculated perpendicular to the strip on both sides and without touching the strip. A positive reaction by toxigenic strains produces a precipitin line at a 45-degree angle to the inoculum streak.

18.

D. Erysipelas results from person-to-person transmission of group A streptococci. Symptoms occur when nasopharyngeal infection spreads to the face. The rare complication of an upper respiratory infection with *Streptococcus pyogenes* is characterized by sensations of burning and tightness at the site of invasion. Erythema associated with this superficial cellulitis rapidly spreads with an advancing elevated margin. *Erysipelothrix rhusiopathiae* causes a similar disease referred to as erysipeloid.

19.

B. Phenylethyl alcohol agar (PEA) is a selective medium for the isolation of gram-positive cocci. Blood agar medium is supplemented with 0.15% phenylethyl alcohol, which is inhibitory to most gram-negative aerobic bacilli. This medium is particularly helpful when a specimen containing gram-positive cocci is contaminated with a *Proteus* spp. due to the inhibition of swarming by PEA.

20.

D. Viridans streptococci are the most common normal flora in upper respiratory cultures. They are opportunistic pathogens with low virulence. Subacute endocarditis is seen in patients with previously damaged heart valves.

21.

B. Whether growing aerobically or anaerobically, streptococci obtain all their energy from the fermentation of sugars to lactic acid. Streptococci are all catalase negative and grow on conventional media such as sheep blood agar. Most are part of the normal flora of human skin, throat, and intestine but produce a wide variety of infections when introduced in tissues or blood.

22.

A. Organisms that synthesize the enzyme catalase are able to protect themselves from the killing effects of H_2O_2 by converting it to H_2O and O_2 . Streptococci are unable to synthesize the heme prosthetic group for this enzyme and are catalase negative. Therefore, they grow better on blood-containing media because of the catalase-like activity of hemoglobin.

23.

D. Streptolysin S is primarily responsible for the beta-hemolysis seen on the surface of a sheep blood agar plate inoculated with a group A streptococcus. Of the two hemolysins secreted by beta-hemolytic group A streptococcus, streptolysin S is stable in the presence of atmospheric oxygen. Streptolysin O is inactivated in the presence of oxygen, and it is best demonstrated when the agar has been stabbed and subsurface hemolysis is revealed.

24.

B. *Streptomyces* are weak pathogens rarely associated with disease. The bacteria normally inhabit the soil. The most common human infection is mycetoma, which is most frequently caused by *S. somaliensis*. Some strains of *Streptomyces* grow better at 25°C than at 35°C.

25.

B. *Erysipelothrix* is a nonmotile, catalase-negative, gram-positive bacillus that often appears as long filaments. Unlike other aerobic gram-positive bacilli, this organism produces H_2S , which can be demonstrated in triple sugar iron agar. Erysipeloid, a skin disease of the hands usually associated with the handling of infected animals, is the human infection produced most commonly by this agent.

26.

D. *Listeria monocytogenes* is motile at room temperature. When inoculated into a semisolid medium, growth away from the stab is characteristic of motility. Motility is generally enhanced just below the agar surface, giving the growth pattern an “umbrella” appearance. *L. monocytogenes* is nonmotile at 35°C.

27.

D. *Rhodococcus equi* is found in soil and commonly produces disease among livestock. These gram-positive bacilli can demonstrate primary mycelia and were formerly in the genus *Nocardia*. This species is characterized by its pink pigmentation on culture media and its inability to ferment carbohydrates.

28.

A. *Streptococcus pneumoniae*, a primary etiologic agent of lobar pneumonia, is an encapsulated, gram-positive, lanceolate diplococcus. Fastidious in its growth requirements, the organism on sheep blood agar produces characteristic alpha-hemolytic colonies, which are convex and often mucoid in appearance and bile soluble. Upon aging, colonies of *S. pneumoniae* undergo autolytic changes. There are approximately 80 types of pneumococci based on specific capsular antigens.

29.

D. Species of the genus *Nocardia* are ubiquitous in the soil and thus characteristically produce exogenous forms of infection as a result of inhalation of contaminated fomites or a traumatic incident with soil contamination. A diagnostic characteristic, depending on the species, is the acid fastness of the filamentous bacilli or coccoid forms. Unlike *Actinomyces* spp., which are catalase-negative, gram-positive, non-spore-forming anaerobic bacilli, *Nocardia* spp. are catalase-positive aerobic organisms. "Sulfur granules" are characteristic of actinomycotic pus and upon examination would reveal non-acid-fast branching filaments.

30.

B. *Enterococcus faecium* is an important agent of human infection. Their differentiation from other enterococcal strains is of importance because of their resistance to most clinically useful antimicrobial agents, including vancomycin. The ability to tolerate a high concentration of salt is characteristic of the clinically significant species of *Enterococcus*. *E. faecium* is PYR positive and is usually nonhemolytic.

31.

D. Viridans streptococci do not produce the enzyme pyroglutamyl aminopeptidase and, therefore, in the PYR test do not produce a positive or red color. The PYR test is used predominantly for the presumptive identification of group A streptococci and *Enterococcus*. *Micrococcus* and *Lactococcus* are known to produce a positive reaction as well, although the reaction may be delayed.

32.

C. *Streptococcus pneumoniae* is a leading cause of lobar pneumonia as well as other serious bacterial infections. The Gram stain smear of clinical specimens can provide a rapid presumptive diagnosis when the characteristic morphology and Gram reaction is observed. The optochin disk test can be performed to presumptively identify this organism. Optochin lyses pneumococci, producing a zone of inhibition around the disk.

33.

D. Bacteriologic cultures of a typical impetigo lesion may yield either a pure culture of *Streptococcus pyogenes* or a mixed culture of *S. pyogenes* and *Staphylococcus aureus*. The thick crust form of impetigo, which is most commonly seen, is primarily caused by *S. pyogenes*. It is the bullous form of impetigo for which *S. aureus* is the etiologic agent. The route of infection is direct inoculation of the causative agents into abraded or otherwise compromised areas of the skin.

34.

C. Identifying characteristics of *Staphylococcus aureus* include the production of the extracellular enzymes coagulase and DNase and its ability to grow in the presence of high salt concentrations. Differential and selective media, such as mannitol salt agar, have been developed for the recovery of this organism. Selective media and rapid identification tests are important for this widely recognized opportunistic pathogen.

35.

A. Group B streptococci (*Streptococcus agalactiae*), unlike other streptococci, can hydrolyze sodium hippurate to benzoic acid and glycine. If glycine is produced, the addition of ninhydrin to the medium will reduce the glycine to produce a purple color. The use of ninhydrin to detect glycine is a sensitive and rapid test of hippurate hydrolysis.

36.

B. *Listeria monocytogenes* and *Streptococcus agalactiae* produce an extracellular factor known as the CAMP factor. The test is performed by making a streak of the test isolate perpendicular to a streak of *Staphylococcus aureus*. A positive CAMP reaction is indicated by a zone of enhanced beta-hemolysis (arrowhead shape) at the point where the zone of hemolysis produced by *S. aureus* joins with that produced by the beta-hemolytic test isolate. Unlike *S. agalactiae*, *L. monocytogenes* is catalase positive.

37.

A. The production of hemolysins and the enzymes coagulase and DNase is associated with the virulence of staphylococci. The coagulase-producing staphylococci are most commonly producers of staphylolysin, which produce beta-hemolysis when the isolate is grown on sheep blood agar. Many factors contribute to staphylococcal virulence by overcoming the host's natural defenses. Endotoxin is found in the cell wall of gram-negative bacteria.

38.

C. The physiology of staphylococci enables them to remain infectious in the environment longer than many other pathogenic bacteria. Staphylococci are somewhat heat resistant and can survive dry conditions. Their high salt tolerance enables strains to grow in salt-preserved

foods and causes cases of food poisoning. Staphylococci, however, cannot resist temperatures as high as 55°C for long periods, and they are not bile resistant. Most species are sensitive to novobiocin.

39.

C. Motility is a key test for the differentiation of *Bacillus anthracis* from other species of *Bacillus*. Suspect *Bacillus* colonies are inoculated in a broth medium and allowed to grow to a visible turbidity. A sample of this actively growing culture should be examined using the hanging-drop technique for motility. *B. anthracis* is nonmotile and can therefore be easily differentiated from commonly encountered motile species.

40.

D. The rash of scarlet fever is a result of the action of an erythrogenic toxin produced by group A streptococci. Because of the rapid diagnosis and treatment of group A streptococci infections, scarlet fever is rare in most developed countries. The other diseases listed do not involve an erythrogenic toxin.

41.

D. Cultures of the tonsillar fossae and posterior pharynx are most commonly obtained in suspected cases of streptococcal pharyngitis. *Streptococcus pyogenes* is most often associated with cases of pharyngitis but is also the agent of scarlet fever and erysipelas in addition to wound infections (e.g., necrotizing fasciitis). Rapid identification of this organism and prompt antimicrobial therapy are required to prevent sequelae (i.e., rheumatic fever and acute glomerulonephritis).

42.

D. Subacute bacterial endocarditis is an inflammation of the lining membrane of the heart, which most often is caused by a member of the viridans group of streptococci. *Streptococcus sanguis* is one of several species that may lodge in an abnormal heart or on valves damaged by previous infection. Viridans streptococci are normal inhabitants of the human upper respiratory tract.

43.

C. *Streptococcus pneumoniae* is most commonly associated with cases of lobar pneumonia. Patients characteristically produce blood-tinged, rust-colored sputum in which the characteristic gram-positive lanceolate diplococci can be found. *S. pneumoniae* forms alpha-hemolytic colonies when grown on sheep blood agar.

44.

A. *Staphylococcus saprophyticus* is recognized as an etiologic agent of uncomplicated cystitis cases in young females. These nonhemolytic, coagulase-negative staphylococci closely resemble *S. epidermidis* on sheep blood agar. Identification of *S. saprophyticus* is facilitated by demonstrating its resistance to novobiocin.

45.

A. Clinical material sent to the laboratory for the recovery of *Corynebacterium diphtheriae* should be inoculated on cystine-tellurite agar plates or Tinsdale medium. On tellurite-containing media, colonies of this pathogen will appear dark-brown to black, which aids in their differentiation. Suspicious colonies should be further tested for their biochemical activity and toxin production.

46.

D. *Bacillus anthracis* is the causative agent of woolsorters disease or the pulmonary form of anthrax. The mode of infection is the inhalation of spores by the patient, usually during the performance of his/her occupation (sheep shearing or processing of animal hair). Prompt diagnosis and treatment of this disease is needed because it is known to progress rapidly to a fatal form of septicemia.

47.

D. *Corynebacterium pseudodiphtheriticum* is morphologically similar to all other members of the genus *Corynebacterium*. They are all gram-positive, non-spore-forming bacilli that characteristically resemble Chinese characters or palisades. These bacteria often stain irregularly and have a pleomorphic club-shaped appearance.

48.

B. *Corynebacterium jeikeium* is a low virulence organism resistant to multiple antimicrobials. Its multiple drug resistance allows it to remain in hospital environments, and it is often cultured from the skin of hospitalized patients. In compromised patients it has been implicated in cases of septicemia, wound infections, and endocarditis in association with intravenous catheter use.

49.

B. In immunocompromised patients, *Nocardia asteroides* can cause invasive pulmonary infection and can often spread hematogenously throughout the body. Lesions in the brain are commonly associated with dissemination and have a poor prognosis. The organism is ubiquitous in nature, and infection is acquired by traumatic inoculation or inhalation.

50.

C. Of the genera listed, only *Leuconostoc* is catalase negative. *Leuconostoc* is vancomycin resistant and associated with infections in hospitalized patients. It has also been linked to septicemias in neonates.

51.

B. *Listeria monocytogenes* is a small, gram-positive bacillus that is actively motile at room temperature. When grown on sheep blood agar, this organism produces small, translucent beta-hemolytic colonies, which may be visually mistaken for beta-hemolytic streptococci. Biochemically *L. monocytogenes* differs from streptococci because it possesses the enzyme catalase.

52.

C. *Staphylococcus epidermidis* is a saprophytic microorganism found on the skin and mucous membranes of humans. This coagulase-negative *Staphylococcus* is seen frequently as a contaminant in blood cultures when proper venipuncture technique has not been used. *S. epidermidis* has been implicated in serious human infections associated with the surgical insertion of prosthetic devices.

53.

A. *Bacillus anthracis* is the etiologic agent of human anthrax that occurs in any of three forms: cutaneous, pulmonary, and gastrointestinal. On Gram stain this organism appears as a large, spore-forming, gram-positive bacillus that characteristically grows in long chains. Colonies on agar plates are large and opaque with fingerlike projections referred to as "Medusa head" forms.

54.

C. Staphylococci colonize various skin and mucosal surfaces in humans. *S. aureus* is carried

as transient flora in the anterior nares. *S. saprophyticus* is less likely found as normal flora and is associated with urinary tract infections. Hospital personnel may harbor resistant strains of *S. aureus*, and person-to-person contact is a substantial infection control concern. Cultures of the anterior nares are recommended when screening for carriers in the hospital environment.

55.

D. The susceptibility of alpha-hemolytic streptococcal isolates to optochin, or ethylhydrocupreine HCl, is a presumptive test for the differentiation of *Streptococcus pneumoniae* from viridans streptococci. Viridans streptococci are typically resistant to this agent and show no zone of inhibition or a zone of less than 10 mm with a 6-mm disk. *S. pneumoniae* characteristically is susceptible and produces a zone of inhibition greater than 14 mm.

56.

D. Solubility of *Streptococcus pneumoniae* colonies by surface-active agents, such as sodium desoxycholate, is a widely used presumptive identification procedure. When a 10% solution of this reagent is applied to test colonies, *S. pneumoniae* will be totally dissolved. Colonies of viridans streptococci typically remain intact when bile is applied.

57.

D. The ingestion of food contaminated with enterotoxin produced by *Staphylococcus aureus* is the most likely cause of the disease in the case described. *S. aureus* multiplies rapidly in improperly stored food. Within a few hours, levels of 10^5 organisms per gram of food can be found. Enterotoxin is elaborated when the organism reaches stationary growth phase. Ingestion of small amounts of toxin results in a rapid onset (1–6 hours) of vomiting and diarrhea as a result of a neural response.

58.

D. Most strains of *Staphylococcus saprophyticus* are resistant to novobiocin. This organism is frequently found in urine culture of young women and may be misidentified as *S. epidermidis*. A 5- μ g disk is used in the test, and a zone of 16 mm or less determines resistance.

59.

B. On serum-cystine-sodium thiosulfate-tellurite medium (Tinsdale medium), *Corynebacterium diphtheriae* is differentiated from other corynebacteria and other bacteria of the respiratory tract by its ability to produce black colonies surrounded by a brown-black halo after 48 hours of incubation. Growth factors needed by *C. diphtheriae* are provided by the addition of the serum. Potassium tellurite is inhibitory to many gram-positive and gram-negative bacteria, but corynebacteria are resistant.

60.

C. *Staphylococcus aureus* is the predominant pathogen involved in joint infections of adults. Bacterial arthritis can occur following infection in other parts of the body or bacteremia. *Streptococcus pyogenes* and *Neisseria gonorrhoeae* each account for a significant number of adult infections, whereas *Streptococcus pneumoniae* and *Haemophilus influenzae* predominate in childhood infections.

61.

B. *Listeria monocytogenes* is a cause of human and bovine abortion. In humans, the mother's symptoms are usually mild, resembling the flu and causing a low-grade fever. The organism can be isolated from aborted fetuses as well as from the maternal placenta. When infection with this etiologic agent is detected early, appropriate therapy can be initiated, which may prevent the death of the fetus.

62.

A. *Bacillus* spp. are gram-positive, spore-forming bacilli widely found in the environment. *Bacillus cereus* is of particular interest as an etiologic agent of human cases of food poisoning. This enterotoxin-producing microorganism is most commonly associated with cases of food poisoning following ingestion of reheated rice served at Asian restaurants.

63.

A. *Bacillus anthracis* infects humans by three routes: respiratory, gastrointestinal, and cutaneous. Malignant pustule is the name given to lesions seen in cutaneous anthrax in humans. The lesion is, however, neither malignant nor a pustule. The disease produces a localized abscess on the skin, which forms a characteristic black eschar surrounded by a red raised ring.

64.

B. Scalded skin syndrome is the dermatitis associated with the effects of the exfoliative toxin produced by strains of *Staphylococcus aureus*. Exfoliatin acts in humans to disrupt the adhesive forces between cells of the stratum granulosum, creating large flaccid bullae. This syndrome occurs primarily in infants and children; the primary infection is usually unrelated to the areas where lesions appear.

65.

B. *Enterococcus faecalis* and *E. faecium* grow in the presence of bile, hydrolyze esculin, and produce acid from glucose in the presence of high salt concentration. These bacteria also express streptococcal group D antigen. The ability to tolerate high salt concentrations differentiates the enterococci from the group D streptococci like the *S. bovis* group.

Aerobic Gram-Negative Bacteria

66.

A. The etiologic agents of brucellosis are the brucellae, which are small, nonmotile, gram-negative coccobacilli that are facultative intracellular parasites. Isolation of these organisms is difficult. In suspected cases, which are generally job related, multiple blood cultures are recommended for optimal recovery of the agent. Bone marrow cultures have been found to be positive when cultures of blood failed to recover the organism.

67.

B. *Klebsiella pneumoniae* is the species most frequently recovered from the vast majority of clinical cases. Members of the genus *Klebsiella* have a capsule and appear mucoid on cultures. This highly encapsulated organism can cause severe pneumonia, nosocomial infections of several types, infantile enteritis, and other extraintestinal infections.

68.

A. Enterotoxins are produced in the intestinal tract and primarily cause diarrhea. The heat-labile enterotoxin of *Escherichia coli*, which resembles cholera toxin, acts to stimulate the enzyme adenylate cyclase. The stimulation of the enzyme adenylate cyclase by the toxin increases the production of cyclic AMP, causing rapid gastrointestinal fluid loss. Diarrhea results following stimulation of the secretion of chloride ions by the cells lining the small intestine.

69.

C. The genus *Neisseria* contains organisms that possess cytochrome oxidase activity. Colonies can be identified by the development of a dark purple color following the application of tetramethyl-*p*-phenylenediamine dihydrochloride. The reaction relies on the property of the molecule to substitute for oxygen as an electron acceptor. In

the presence of the enzyme and atmospheric oxygen, the molecule is oxidized to form indophenol blue.

70.

A. *Shigella* has a low infecting dose and has been reported to cause outbreaks in daycare centers and can be spread to family members. These organisms are found in humans only at the time of infection; they are not part of the normal flora. Transmission is by the fecal-oral route, typically by ingestion of contaminated foods or water.

71.

A. *Chryseobacterium* spp. are ubiquitous in the environment and are especially associated with moist soil and water. *Chryseobacterium* (formerly *Flavobacterium*) *meningosepticum*, a known nosocomial pathogen, has been implicated in outbreaks of meningitis in hospitals and is associated with the use of contaminated respiratory therapy equipment. Adult human infections are rare; these opportunistic microorganisms occur primarily in immunocompromised patients.

72.

D. *Shigella dysenteriae*, the type species of the genus, is a causative agent of bacillary dysentery. Differential and selective media for the recovery of enteric pathogens from stool samples would demonstrate *Shigella* species as H₂S negative, non-lactose-fermenting, gram-negative bacilli. Further biochemical testing would generally show these organisms to be unable to use citrate as their sole carbon source, unable to decarboxylate the amino acid lysine, and urease negative.

73.

A. *Aeromonas hydrophila* is typically found in fresh water and has been implicated in human infections. Growth on MacConkey agar and a positive oxidase reaction are characteristic of this organism. A positive oxidase reaction differentiates this organism from all of the *Enterobacteriaceae*, except the recently added *Plesiomonas shigelloides*. On sheep blood agar, many strains of *Aeromonas* produce beta-hemolysis.

74.

B. A highly selective medium, thiosulfate-citrate-bile salt-sucrose (TCBS) is used for the isolation of *Vibrio* spp. Species able to ferment sucrose, such as *V. cholerae*, produce yellow colonies. Non-sucrose-fermenting organisms produce green colonies.

75.

A. The K (capsule) antigen surrounds the bacterial cell and masks the somatic antigens of the cell wall, which are used to group members of the *Enterobacteriaceae*. These heat-labile antigens can be removed by heating a suspension of the culture at 100°C for 10–30 minutes. Antisera that contain K antibody can be used to demonstrate the presence of the capsular antigens.

76.

B. *Burkholderia pseudomallei* is the causative agent of melioidosis. The bacterium is found in soil and water in subtropical areas of Southeast Asia and Australia. Melioidosis exhibits several forms, from skin abscesses to abscess formation in internal organs.

77.

C. *Francisella tularensis* requires cysteine or cystine for growth. Glucose-cysteine with thiamine and cystine heart media are commercially available for suspected cases of tularemia. They

both require the addition of 5% sheep or rabbit blood. Buffered charcoal yeast extract also supports the growth of *F. tularensis*, a medium generally used by clinical laboratories for the cultivation of *Legionella* spp.

78.

B. Decubitus ulcers frequently contain normal intestinal flora. The biochemical results are characteristic of *Escherichia coli*. *E. coli* is associated with a variety of diseases; it is the predominant organism associated with cases of neonatal meningitis, septicemia, cystitis, appendicitis, and endocarditis.

79.

D. *Pseudomonasa aeruginosa* is the most commonly encountered gram-negative species that is not a member of the family *Enterobacteriaceae*. It is ubiquitous in nature and is found in homes and hospitals. It is an opportunistic pathogen responsible for nosocomial infections.

80.

A. *Capnocytophaga* spp. are fermentative gram-negative bacteria that inhabit the oral cavity of humans. These organisms have been identified as a cause of disease in the oral cavity, and in compromised hosts they have been implicated in systemic disease isolated from cerebrospinal fluid, pleural fluid, and pulmonary secretions. The gliding motility is best observed during the log phase of growth and can be demonstrated by darkfield microscopy and on sheep blood agar by the production of concentrically spreading growth around primary colonies.

81.

C. *Vibrio vulnificus* is implicated in wound infections and septicemia. The organism is found in brackish or salt water. Ingestion of contaminated water or seafood is the typical mode of transmission. Wound infections are associated with contamination at the site with organisms in water.

82.

D. The Waterhouse-Friderichsen syndrome of disseminated intravascular coagulation occurs in cases of fulminant meningococcemia—*Neisseria meningitidis* septicemia. Invasion of the circulatory system by *N. meningitidis* may produce only a transient bacteremia or meningitis or may go on to cause a rapidly fatal infection. In cases of meningococcemia with intravascular coagulation, acute adrenal insufficiency due to hemorrhage into the adrenal gland may result.

83.

B. Before the development of an effective vaccine, the strain of *Haemophilus influenzae* found to be implicated in the majority of cases of bacterial meningitis in children 1–6 years of age was serotype B. This serotype is surrounded by a weakly immunogenic polyribitol phosphate capsule. The widespread use of *Haemophilus influenzae* type b (Hib) vaccine beginning in 1985 has significantly reduced the incidence of invasive *H. influenzae* type b disease.

84.

A. Unlike other salmonellae, *Salmonella* Typhi produces only a small amount of hydrogen sulfide, produces no gas from glucose, is citrate negative, and possesses a capsular antigen (Vi). Identification of *Salmonella* Typhi, the etiologic agent of typhoid fever, may be delayed if laboratory professionals do not have a good appreciation of its atypical characteristics. It is also important to note that the bacilli appear in the

patient's circulatory system several days before a stool culture will be positive.

85.

A. Chancroid or soft chancre is caused by *Haemophilus ducreyi*, a small, gram-negative coccobacillus. Painful genital lesions and painful swelling of the inguinal lymph nodes characterize the disease. The incubation period following contact with an infected person ranges from 1 to 5 days, after which the patient notes the painful, round, nonindurated primary lesion on the external genitalia. Signs of regional lymphadenitis appear in about one-half of the cases a few days after the appearance of the primary lesion.

86.

D. Pseudomonads are ubiquitous microorganisms generally associated with moist environments. Cases have been increasing as the popularity of health spas increases. In some cases the pattern of dermatitis caused by these organisms matches the areas covered by the individual's swimsuit. When not properly maintained, whirlpools create a favorable environment for the growth of these organisms.

87.

B. *Campylobacter jejuni* rivals *Salmonella* as the most common bacterial cause of diarrheal disease in humans. *Campylobacter* enterocolitis is characterized by fever, bloody diarrhea, and abdominal pain. Special selective culture media and incubation under a microaerophilic atmosphere at 42°C are required for the recovery of this organism from clinical samples.

88.

D. Screening procedures for the recovery of the enteric pathogen *Salmonella* rely heavily on differential media, which indicate lactose fermentation and the production of H_2S . Isolates of *Shewanella putrefaciens* recovered from stool samples on a medium such as Hektoen enteric agar would resemble *Salmonella* in that the organism is not able to ferment lactose and does produce a significant amount of H_2S . However, unlike *Salmonella*, some strains of *S. putrefaciens* will produce acid from sucrose, forming yellow colonies on Hektoen enteric agar.

89.

A. Whooping cough, or pertussis, is caused by *Bordetella pertussis*, a minute, encapsulated, nonmotile, gram-negative, pleomorphic bacillus. The best identification method is the polymerase chain reaction. Regan-Lowe medium is recommended for the isolation of this agent.

90.

B. *Neisseria gonorrhoeae* is a primary pathogen of the urogenital tract. It is an important cause of sexually transmitted diseases. Surface structures such as pili aid in attachment to mucosal epithelial cells and invasion of submucosa to produce infection.

91.

D. Most serotypes of *Salmonella* produce hydrogen sulfide in triple sugar iron agar. As seen in Color Plate 27 ■, they demonstrate a positive lysine and a negative urease reaction, which differentiates them from *Proteus* spp., which also produce H_2S . It is important to be able to quickly differentiate those organisms resembling *Salmonella* from other H_2S -producing organisms such as *Citrobacter freundii* and *Edwardsiella tarda*.

92.

A. *Neisseria meningitidis* and *N. gonorrhoeae* are most commonly described as having a “kidney bean” cellular morphology. Occasionally some *Moraxella* spp. will exhibit this morphology. These gram-negative coccil organisms appear characteristically as diplococci, with the paired cells having adjacent walls that are flattened. *Neisseria* are important human pathogens.

93.

C. *B. cepacia* is the most common *Burkholderia* spp. in clinical specimens. *P. aeruginosa* is the most common gram-negative bacillus that is not in the family *Enterobacteriaceae* and *Stenotrophomonas maltophilia* the second most common. *B. mallei* has not been isolated recently in the U.S.

94.

D. Ophthalmia neonatorum, a form of conjunctivitis, is associated with *Neisseria gonorrhoeae*. The infection is transmitted to the newborn by the mother as it passes through the birth canal. The use of an ophthalmic solution of erythromycin is recommended for the prevention of this form of conjunctivitis.

95.

B. *Neisseria lactamica* is part of the normal nasopharyngeal flora of humans. In the laboratory this agent may be mistakenly identified as *Neisseria meningitidis*, an organism of significant pathogenicity. Differentiation of these two species is easily accomplished by demonstrating the fermentation of lactose or an ONPG (*o*-nitrophenyl-beta-galactopyranoside) positive test.

96.

A. *Haemophilus aegyptius* is the causative agent of “pinkeye.” This form of conjunctivitis is highly contagious and is frequently seen in children attending daycare centers. The agent is an aerobic gram-negative bacillus that is nonmotile and requires both hemin (X factor) and nicotinic adenine dinucleotide (NAD, V factor) for growth.

97.

A. *Hafnia* is a member of the family *Enterobacteriaceae*. *Hafnia alvei* is the only species in the genus. The characteristics of this organism are positive motility and lysine, ornithine, ONPG, and KCN reactions.

98.

B. *Acinetobacter* spp. are opportunistic pathogens for humans and are important causes of nosocomial infections. They are oxidase negative and will grow on most laboratory media, including MacConkey agar. *Acinetobacter* spp. are nonfermenters, but *A. baumannii* can form pink to purple colonies on MacConkey agar that can be mistaken for lactose fermentation. Many strains of *A. baumannii* will oxidize glucose.

99.

B. Pontiac fever is caused by *Legionella pneumophila*, as is Legionnaires disease, but it is not as serious an infection. This febrile illness is characteristically self-limited and does not demonstrate significant pulmonary symptoms. The incubation period, unlike that for Legionnaires disease, is short, followed by symptoms of malaise, muscle aches, chills, fever, and headache.

100.

A. *Brucella* spp. are fastidious, gram-negative, coccobacillary organisms. They are predominantly animal pathogens, but occasionally produce disease in humans. The usual specimens for

recovery of *Brucella* are blood and bone marrow, with the latter considered the more sensitive.

101.

A. *E. coli* O157:H7 produces a toxin similar to Shiga toxin produced by *Shigella dysenteriae*. It is most commonly transmitted by ingestion of undercooked ground beef or raw milk. Hemorrhagic colitis is characteristic of infection, but infection can also lead to hemolytic uremic syndrome resulting from toxin-mediated kidney damage.

102.

A. *Aeromonas* spp. are found in bodies of fresh water and salt water that can be flowing or stagnant and contaminated with sewage. These organisms are known as one of the animal pathogens that cause “red leg disease” in frogs. The largest number of human cases occurs between May and November and seems to be highly associated with exposure to water or soil.

103.

C. *Campylobacter jejuni* are small, curved, motile gram-negative rods that are hippurate hydrolysis positive. They are found in the gastrointestinal tract of a variety of animals. Campy agar is used for isolation from stool and is incubated at 42°C under microaerophilic conditions (10% CO₂, 5% O₂ with balance N₂) for 72 hours.

104.

C. *Hafnia alvei* is a member of the family *Enterobacteriaceae* and is oxidase negative. It has been isolated from a variety of clinical specimens but is generally considered clinically insignificant. *Aeromonas*, *Chryseobacterium*, and *Vibrio* are all oxidase positive.

105.

A. *Neisseria gonorrhoeae* is the causative agent of gonorrhoea and is very sensitive to drying, temperature variations, and fatty acids in clinical material. *N. gonorrhoeae* will grow on chocolate agar but not sheep blood agar. Incubation under CO₂ is required for recovery, and selective media like Thayer-Martin are recommended.

106.

D. The most commonly used method for serogrouping *Shigella* is based on the somatic oligosaccharide or O antigens. The O antigens are also used to serogroup *Escherichia coli* and *Salmonella*. Because *Shigella* spp. are non-motile, the H or flagella antigens cannot be used; however, H antigens are used for *E. coli* and *Salmonella*. The capsule (K) antigens are used to serogroup *Klebsiella pneumoniae*.

107.

C. *Vibrio cholerae* produces an exotoxin that causes infected individuals to lose massive amounts of fluids. Severe dehydration is usually the cause of death in untreated patients. Proper therapy begins with intravenous fluids to restore the patient's water volume and electrolyte balance. The microorganism does not invade the intestinal mucosa but is attached to the surface of enterocytes. The other bacteria listed produce toxins or invasive diseases that often produce inflammation resulting in bloody diarrhea.

108.

C. *Vibrio parahaemolyticus* is classified as a halophilic *Vibrio* sp. requiring increased osmotic pressure, in the form of salt, for growth. This makes routine biochemical test media less than optimal because of their low NaCl content. Growth in the presence of 1% NaCl but no growth in media without the added Na⁺ is the test for the

differentiation of halophilic organisms. Marine water is the normal habitat of most *Vibrio* spp.

109.

C. *Brucella* spp. are hazardous, especially in aerosol-generating procedures. It is important for the laboratory to be notified whenever brucellosis is suspected. Most laboratories send isolates to a reference laboratory for confirmation or definitive identification because they lack specialized media and containment facilities.

110.

B. *Helicobacter pylori* is found in the human gastric mucosa colonizing the mucous layer of the antrum and fundus but does not invade the epithelium. Approximately 50% of adults over the age of 60 years are infected, with the incidence of gastritis increasing with age. *H. pylori* has been cultured from feces and dental plaque, supporting the theory of a fecal-oral or oral-oral route of transmission.

111.

A. *Acinetobacter* is widely distributed in nature and commonly colonizes hospitalized patients. Infection occurs mainly in compromised hosts. Its resistance to many of the commonly used antimicrobial agents limits the selection of therapeutic agents.

112.

D. *Vibrio parahaemolyticus* is found in brackish or salt water. The mode of transmission is the ingestion of contaminated water or seafood. *V. parahaemolyticus* is halophilic.

113.

D. Organisms biochemically resembling *Salmonella* are typically tested using a polyvalent antiserum composed of antibodies against the commonly isolated strains, including antisera against the Vi antigen. The Vi antigen is a heat-labile capsular antigen associated with *Salmonella* Typhi. After heating a *Salmonella* Typhi suspension, the Vi antigen has been removed, and the organism can now react with the somatic grouping antisera. *Salmonella* Typhi demonstrates a positive agglutination reaction in D-grouping sera.

114.

B. *Campylobacter fetus* subsp. *fetus* is occasionally implicated in human disease. This organism, unlike *C. jejuni*, is characterized as producing extraintestinal symptoms. Those persons most at risk of infection are those with preexisting disease who are in a debilitated condition.

115.

A. *Shigella sonnei* is a group D *Shigella* and is characterized by its ability to ferment lactose. *S. sonnei* is ONPG positive but is a delayed lactose fermenter. This is the most commonly isolated species of *Shigella* in the U.S. The genus *Shigella* is characterized biochemically by being negative for citrate, urease, motility, and lysine decarboxylation.

116.

A. The Voges-Proskauer (VP) test is a broth test that detects the presence of acetoin from the metabolism of glucose in the medium. A red color indicates a positive reaction. The most common clinical isolates from the genera *Providencia*, *Escherichia*, *Salmonella*, and *Proteus* are generally VP negative, whereas most members of the *Klebsiella*, *Enterobacter*, and *Serratia* are positive. *Enterobacteriaceae* that are VP positive are typically methyl red negative.

117.

C. Pathogenic mechanisms of *V. cholerae* include adherence to enterocytes via pili, motility, enzymes such as protease and mucinase, and the production of an enterotoxin. Epidemic strains colonize the small intestine where they elaborate cholera toxin, an enterotoxin. Disease is produced when the enterotoxin stimulates the secretion of large volumes of fluids into the intestinal lumen.

118.

A. Classic epidemic strains of *Vibrio cholerae* are included in the antigenic O group 1. The Ogawa and Inaba strains are considered the predominant epidemic strains. In the last few years the strain O139 has also been associated with outbreaks of cholera.

119.

D. *Brucella* spp. are small, gram-negative intracellular parasites implicated in zoonotic infection of humans. Brucellosis presents as an undulant febrile illness. In the U.S., disease caused by *Brucella* sp. is mainly job related or involves food or animal associations, such as in hunters or those who drink raw milk.

120.

A. *Escherichia coli* is a lactose-fermenting member of the family *Enterobacteriaceae*. Various selective and differential agars are available for the differentiation of lactose fermenters from those that do not degrade lactose. In some media H_2S production may be demonstrated. Isolates of *E. coli* would produce yellow colonies at 24 hours on xylose-lysine-desoxycholate (XLD) agar. Non-lactose fermenters such as *Shigella* would produce red colonies on XLD agar. On MacConkey agar, lactose fermenters produce pink colonies; on Hektoen enteric agar, colonies would be orange. Most strains of *E. coli* are beta-hemolytic on sheep blood agar.

121.

A. *Yersinia pestis* is the causative agent of plague. The organism is endemic in rodents and is transmitted to humans by the rat flea. This oxidase-negative organism, unlike other *Yersinia* spp., is nonmotile at 20–25°C. It is also negative for H₂S and urease.

122.

B. Motility can be important in the identification of microorganisms. Of the *Enterobacteriaceae*, the genera *Klebsiella* and *Shigella* are characteristically nonmotile, as is *Tatumella*. Motility of the *Enterobacteriaceae* can normally be detected by the use of a semisolid motility medium, which is grossly observed for the determination of motility. The hanging-drop method is perhaps the most accurate means of detecting motility of nonfermentative microorganisms.

123.

C. Pneumonic legionellosis and the nonpneumonic illness known as Pontiac fever are the two clinical forms of disease caused by *Legionella pneumophila*. The optimal temperature for cultivation is 35°C, and cold enrichment is not appropriate. Direct fluorescent antibody is often used diagnostically, and erythromycin is the drug of choice for therapy.

124.

B. *Klebsiella granulomatis*, formerly *Calymmatobacterium granulomatis*, is the etiologic agent of the sexually transmitted disease granuloma inguinale. It is a pleomorphic, gram-negative, encapsulated bacillus, although it does not Gram stain well. First seen as inclusions (Donovan bodies) in mononuclear cells from genital ulcers stained with the Giemsa or Wright stain, these organisms are extremely difficult to recover.

125.

D. When streaked on DNase test medium, colonies of *Staphylococcus aureus* and *Serratia marcescens* will demonstrate a positive reaction for DNase activity. Inoculated plates are incubated 18 to 24 hours, after which the plates are flooded with a 0.1% solution of HCl. DNase-producing organisms are differentiated by the development of a clear zone in the agar around the colonies.

126.

D. Citrate utilization is a common test used for the differentiation of members of the family *Enterobacteriaceae*. Both *Escherichia coli* and *Shigella boydii* are incapable of using citrate as the sole source of carbon as an energy source. Organisms such as *Citrobacter freundii*, *Enterobacter aerogenes*, and *Serratia marcescens* are able to grow on citrate agar and produce a color change from green to blue in the medium. Many *Enterobacteriaceae* that are Voges-Proskauer positive are also citrate positive.

127.

B. *Neisseria gonorrhoeae* is a fastidious organism requiring the addition of serum or blood to the culture media in order to grow. A selective medium such as modified Thayer-Martin or GC-Lect should be used for primary isolation, especially from sites that may be contaminated with normal flora. Collection and processing of specimens must be done under optimal conditions, because this organism is sensitive to drying and low temperatures.

128.

A. The indole reaction is a widely used method for differentiating lactose-positive *Escherichia coli* from other members of the family *Enterobacteriaceae*. Organisms such as *E. coli*, which possess the enzyme tryptophanase, are able to metabolize the amino acid tryptophan with the production of indole, pyruvic acid, and ammonia. Indole represents the “T” in the IMViC reactions, a battery of tests used for the identification of the *Enterobacteriaceae*.

129.

A. *E. coli* produces an acid over acid (A/A) reaction on TSI agar that indicates that glucose and either lactose or sucrose or both have been fermented. Bacteria that ferment lactose or sucrose produce large amounts of acid in the medium. The enteric pathogens *Salmonella* and *Shigella* can be ruled out when such a reaction is observed, because they are generally not able to use either lactose or sucrose within 18 hours.

130.

A. Pyocyanin is the nonfluorescent, blue-green, diffusible pigment produced by *Pseudomonas aeruginosa*. It is the only bacterium able to produce this pigment. Pyocyanin mixes with the yellow pigment fluorescein to turn culture media green. Most *P. aeruginosa* strains can be identified presumptively by their characteristic grape-like odor, colony morphology, and blue-green pigment.

131.

C. Color Plate 28 ■ is a Gram stain of a cerebrospinal fluid specimen revealing many white blood cells. All of the bacteria listed are fastidious; however, *Haemophilus influenzae* would be expected to grow on chocolate agar but not sheep blood agar (SBA). *Neisseria meningitidis*, also an important cause of meningitis, would be

expected to grow on SBA incubated in CO₂. *Bordetella parapertussis* and *Brucella* sp. would likely grow on both SBA and chocolate agar, and both are uncommon isolates.

132.

D. *Campylobacter jejuni* is an important human pathogen most commonly associated with cases of bloody diarrhea, fever, and abdominal pain in humans. Special handling of cultures suspected to contain this organism is required for optimal recovery. Cultures should be incubated at 42°C in a microaerophilic atmosphere and examined at 24 and 48 hours for spreading nonhemolytic colonies, which may be slightly pigmented. Wet mounts demonstrate the typical “darting” motility of this isolate.

133.

C. *Moraxella catarrhalis* possesses the enzyme indophenol oxidase. When a 1% solution of tetramethyl-*p*-phenylenediamine (oxidase reagent) is applied to colonies of these organisms, the colonies turn a purple color, which rapidly darkens. The other species listed are oxidase negative.

134.

C. *Cardiobacterium hominis* is a rare pathogen that is recovered predominantly from cases of endocarditis. It is characterized as a fermentative, gram-negative bacillus that is nonmotile, catalase negative, oxidase positive, and weakly indole positive. *C. hominis* will grow on sheep blood agar, but growth is enhanced by the addition of yeast extract to media.

135.

D. *Vibrio vulnificus* is a halophilic lactose-fermenting organism. The isolate is associated with two distinct clinical conditions: primary septicemia and wound infection. Septicemia with this organism appears to be correlated in most cases with preexisting hepatic disease. Septicemia due to *V. vulnificus* characteristically produces a fulminant disease with a high mortality rate. Wound infection with this organism is usually associated with trauma and contact with a marine environment.

136.

C. Members of the tribe *Proteae* are characteristically positive for phenylalanine deaminase (PDA). This includes *Proteus vulgaris*, *Providencia stuartii*, and *Morganella morganii*. *Tatuelia ptyseos* belongs to the family *Enterobacteriaceae* and is also PDA positive.

137.

A. The water-diffusible yellow pigment fluorescein (pyoverdine) is produced by members of the *Pseudomonas* fluorescent group, which includes *Pseudomonas aeruginosa*, *P. fluorescens*, and *P. putida*. The production of pyoverdine can be detected when a culture of the organism is exposed to a short-wavelength, ultraviolet light source. The production of fluorescent pigments is dependent upon nutritional factors; therefore, special media, such as cetrinide, should be used when trying to detect the pigment. Cationic salts such as magnesium sulfate intensify luminescence.

138.

C. *Kingella* spp. are gram-negative bacilli or coccobacilli that may appear in short chains. *K. denitrificans* can be isolated from the human upper respiratory tract, will grow on modified Thayer-Martin agar, and is oxidase-positive. The growth of this organism is inhibited by

MacConkey agar, and growth is poor on triple sugar iron agar.

139.

B. Chocolate agar is the preferred culture medium for *Haemophilus*. Unlike 5% sheep blood agar, it provides both hemin (X factor) and NAD (V factor) required for growth. *H. ducreyi* grows best in a special medium, Mueller-Hinton-based chocolate agar, supplemented with 1% IsoVitaleX and 3 µg/mL of vancomycin. *Haemophilus* spp. are obligate parasites of animals and are found primarily in the upper respiratory tract and oral cavity.

140.

A. *Legionella pneumophila* requires the use of special laboratory media for cultivation and does not stain well by the conventional Gram stain. Most *Legionella* spp. are motile, are biochemically inert, and autofluoresce. The primary mode of transmission is by the airborne route, usually in association with an environmental source of bacteria.

141.

C. Tissue samples from the lower respiratory tract (lung biopsy) have the greatest yield of positive cultures for *Legionella pneumophila*. However, these specimens require invasive procedures and are not commonly performed. Cultures of lower respiratory tract specimens, such as bronchial wash and expectorated sputum, are appropriate for the isolation of *L. pneumophila*. The bacteria are seldom recovered from blood specimens.

142.

C. The diagnosis of pertussis, or whooping cough, is confirmed by culture. Regan-Lowe, a charcoal-based medium, provides the best results. Cephalixin is often added to make the medium selective. Isolation of the etiologic agent is best done within the first week of the illness. Modified Skirrow's medium is a primary plating media for *Campylobacter* spp.

143.

C. *Enterobacter sakazakii* produces a yellow pigment that aids in its presumptive identification. Some strains of *E. cowanii* are also pigmented. *E. sakazakii* is an occasional clinical isolate that has been linked to respiratory tract infections and wounds.

144.

C. *Proteus mirabilis* is commonly associated with urinary tract infections as well as infections in other parts of the body. It is a motile organism that characteristically swarms across the surface of sheep blood agar plates. Members of the genus *Proteus* are characteristically rapidly urea positive, lactose negative, and phenylalanine deaminase positive.

145.

D. *Acinetobacter baumannii* is not able to reduce nitrate. This species will oxidize but not ferment glucose. *Acinetobacter* spp. are able to grow on MacConkey agar, and they are oxidase negative, nonmotile, and characteristically resistant to penicillin.

146.

D. *Eikenella corrodens* is a facultatively anaerobic gram-negative bacillus that requires hemin in the culture medium to grow aerobically. This organism, which is a part of the normal indigenous flora of humans, is seldom found in pure culture. It is commonly associated with polymicrobial

infections following bite or clenched-fist wounds. Infections of the face and neck may also involve this organism, which produces pitting of the agar on which it is isolated.

147.

C. In the genus *Pasteurella*, *P. multocida* is the species commonly recovered in clinical specimens. This gram-negative coccobacillus is a normal inhabitant of the oral cavity of domestic animals. Humans most often become infected from a bite or scratch of a cat or dog, which produces a rapidly progressing, painful, suppurative wound infection. Penicillin is an effective drug for the treatment of *Pasteurella* infections.

148.

D. *Legionella pneumophila* is able to hydrolyze hippurate. *L. pneumophila* will also autofluoresce. Although most of the studies done on legionellosis are based on this species, *L. pneumophila* is not the only one associated with human disease.

149.

D. *Haemophilus influenzae* biogroup aegyptius causes a conjunctivitis followed by invasive disease known as Brazilian purpuric fever. The bacterium requires both X and V factors and is therefore negative for delta-aminolevulinic acid (ALA). It resembles *H. influenzae* biotype III in that it is indole negative, urease positive, and ornithine decarboxylase negative.

150.

B. *Kingella denitrificans* is most often associated with endocarditis. It is morphologically similar to *Neisseria gonorrhoeae* both on Gram stain and colonies on culture media. Confusion is further compounded by its ability to grow on modified Thayer-Martin medium and its positive oxidase and glucose reaction. The ability of *K. denitrificans* to reduce nitrates is a key test for its differentiation from *N. gonorrhoeae*.

151.

B. *Plesiomonas* was previously in the family *Vibrionaceae*. Based on nucleic acid and antigenic studies, it was recently moved to the family *Enterobacteriaceae*. *P. shigelloides* is the only species in this genus. Infection in humans has manifested mainly as diarrheal diseases, and a waterborne mode of transmission is often the source.

152.

C. The recalls described illustrate the ubiquitous nature of *Pseudomonas aeruginosa* in the environment and its resistance to many disinfectants. In addition, the bacterium has minimal nutritional requirements and the ability to tolerate a wide range of temperatures (4–42°C). *P. aeruginosa* is an opportunistic pathogen commonly associated with hospital-acquired infections.

153.

B. Ingestion of contaminated unpasteurized (raw) milk or cheese is one of the primary routes of infection. Brucellosis is found worldwide, and symptoms vary from asymptomatic to a debilitating systemic infection. Only four of the six species are typically pathogenic for humans: *B. abortus*, *B. melitensis*, *B. suis*, and *B. canis*.

154.

D. The production of DNase, lipase, and gelatinase differentiates the genus *Serratia* from other *Enterobacteriaceae*. *Serratia* spp., especially *S. marcescens*, have a close association with nosocomial infections. *Serratia* can produce severe infections such as septicemia and meningitis and are frequently difficult to eradicate because of the characteristic antimicrobial-resistant strains found in the hospital environment.

155.

A. *Haemophilus ducreyi* is the causative agent of chancroid, a serious sexually transmitted disease. The disease is more prevalent in the tropics than in temperate parts of the world. The bacteria produce buboes in the groin and can cause a septicemia.

156.

D. Posterior nasopharyngeal cultures are recommended for the recovery of *Bordetella pertussis* in suspected cases of pertussis (whooping cough). Swabs of the nasopharynx are inoculated on the selective agar Regan-Lowe. Cephalixin is added to the culture medium to inhibit the growth of contaminating indigenous flora.

157.

C. *Gardnerella vaginalis* is associated with cases of bacterial vaginosis (BV) formerly called “nonspecific vaginitis.” Although *G. vaginalis* is probably not involved in the pathogenesis of BV, its presence in high numbers is considered a presumptive diagnosis. These small, gram-negative bacilli are frequently seen in great numbers on the surface of epithelial cells (“clue cells”) taken from the vagina.

158.

B. Cultures of blood and bone marrow are the recommended specimens for the isolation of *Brucella* spp. Inoculation of a blood culture bottle for a continuous monitoring system is the most sensitive recovery method. The lysis-centrifugation method (Isolator®, Wampole Laboratories) is more sensitive than a biphasic culture bottle.

159.

C. Mesenteric lymphadenitis is one of the common manifestations of human *Yersinia pseudotuberculosis* infections. Symptoms produced by this agent closely resemble those of acute appendicitis. This gram-negative coccobacillus grows well on routine culture media and has an optimal growth temperature of 25–30°C.

160.

D. Cefsulodin-irgasan-novobiocin (CIN) agar is recommended for the primary isolation of *Yersinia* and *Aeromonas*. *Y. enterocolitica* produces “bull’s-eye” colonies at 48 hours; colonies show a dark red center surrounded by a translucent border. This is a selective and differential agar that suppresses the growth of normal fecal flora and differentiates colonies of *Y. enterocolitica*.

161.

B. *Gardnerella vaginalis* is associated with bacterial vaginosis, but cultures are not recommended for diagnosis. Many women carry *G. vaginalis* as normal vaginal flora; therefore, the isolation of the organism may not be clinically significant. The disease can be diagnosed by detecting “clue” cells, vaginal epithelial cells with gram-variable bacilli attached to their surface.

162.

B. *Legionella pneumophila*, the causative agent of Legionnaires disease, can be recovered from respiratory tract secretions. The bacterium is fastidious and, like *Francisella tularensis*, requires cysteine or cystine for growth. The culture medium most commonly recommended is buffered charcoal yeast extract (BCYE) agar, which is incubated in a moist chamber at 35°C. Growth on this medium may not be visible for 3 to 4 days, after which further identification procedures may be carried out.

163.

D. Swimmer’s ear is a form of external otitis common to persons who swim and fail to completely dry their ear canals when they get out of the water. The organism most commonly associated with this condition is *Pseudomonas aeruginosa*. It is an organism known to be an opportunistic pathogen and one that favors a watery environment.

164.

B. Traveler’s diarrhea is caused by strains of toxin-producing invasive or enteropathogenic *Escherichia coli*. Enterotoxigenic *E. coli* can produce one or two exotoxins: one is heat stable and one is heat labile. Contaminated food products and water in foreign countries seem to be the major vehicle for human infection with these agents.

165.

A. Fresh isolates of *Campylobacter jejuni* on Gram stain characteristically reveal a “gull-wing” appearance. These gram-negative bacilli are motile with a typical darting pattern on wet mounts. They stain poorly using the Gram stain method, and it is recommended that carbolfuchsin or basic fuchsin be substituted for the counterstain safranin.

166.

C. *Neisseria gonorrhoeae* is said to resemble a kidney bean on Gram stain because of its characteristic gram-negative diplococcal morphology in which the adjacent sides are flattened. Typically these organisms are found intracellularly when direct smears of clinical material are examined. Smears from the female genital tract must be interpreted with caution, however, because other normal flora microorganisms are morphologically similar.

167.

A. *Salmonella* is urease negative and H₂S positive. The negative urease reaction would help to differentiate it from an H₂S-positive *Proteus* spp. *Yersinia enterocolitica* and *Y. pseudotuberculosis* are both urease positive. A rapid positive urease is a key test for the identification of *H. pylori*.

168.

A. *Neisseria gonorrhoeae* is identified in the clinical laboratory by its ability to ferment only glucose. The diagnosis of the sexually transmitted disease caused by this agent can be definitively made only by the isolation and identification of *N. gonorrhoeae* in the clinical laboratory. Morphologically, all members of the genus are alike, and all are oxidase positive, which makes definitive identification procedures necessary. Nucleic acid amplification tests are also used frequently to diagnose gonorrhea.

169.

C. *Neisseria meningitidis* is a human pathogen most commonly associated with meningitis. These oxidase-positive, gram-negative diplococci are identified either by fermentation tests or serologic methods that use specific antisera. *N. meningitidis* ferments both glucose and maltose.

170.

A. *Bartonella bacilliformis* is the causative agent of Oroya fever and verruga peruana. It is a pleomorphic, gram-negative rod that is an intracellular parasite of red blood cells and can be cultured from blood in the acute stage of the disease. The disease is rare and occurs primarily in South America.

171.

D. *Serratia liquefaciens*, as its name implies, is able to liquefy gelatin. This bacterium is not thought to be a primary pathogen and is only

rarely isolated from clinical specimens. The positive arabinose reaction is one way of differentiating this isolate from *Serratia marcescens*.

172.

C. *Proteus vulgaris* is able to deaminate phenylalanine. The test is performed by inoculating the isolate on a slant of medium containing phenylalanine and, after incubation, adding a 10% solution of ferric chloride. A dark green color on the slant after addition of the reagent is a positive result.

173.

B. *Klebsiella* spp. are all nonmotile, which aids in their identification. *Klebsiella* spp. produce a capsule resulting in mucoid colonies. *Shigella*, another genus in the family *Enterobacteriaceae*, is also nonmotile.

174.

A. *Chromobacterium violaceum* is a motile, gram-negative bacillus found in soil and water that can be pathogenic for humans. The production of a non-water-soluble violet pigment by these organisms aids in their identification. *Chromobacterium* is catalase and oxidase positive and generally attacks carbohydrates fermentatively.

175.

B. *Pasteurella multocida* is the species in the genus most often encountered in the clinical laboratory. It is normal oral flora in animals, not humans, and it is an opportunistic pathogen. The mode of transmission generally involves traumatic inoculation of the organism through the skin. *P. multocida* grows on sheep blood agar but not on MacConkey agar.

176.

C. *Pseudomonas aeruginosa* has not only a characteristic grapelike odor but also a blue-green color. These oxidative, motile organisms are oxidase positive and are able to grow at 42°C. In humans these opportunistic organisms cause many types of infections, but they are primarily associated with burn wound infections.

177.

A. *Serratia marcescens* is a chromogenic member of the family *Enterobacteriaceae*. *S. marcescens* is the most clinically significant of the genus and is frequently involved in nosocomial infection. The red pigment produced is not water soluble and is demonstrated more readily by incubation at room temperature than at 35°C.

178.

C. *Aeromonas* can be differentiated from many other fermentative gram-negative bacilli, such as the *Enterobacteriaceae*, in that they are oxidase positive. Isolates are ONPG and catalase positive. On sheep blood agar medium, colonies are beta-hemolytic. *A. hydrophila* is found in soil and water and has been isolated from a variety of human infections.

179.

A. *Edwardsiella tarda* is a motile member of the family *Enterobacteriaceae* and as such is characteristically peritrichously flagellated. These organisms are infrequently isolated in the clinical laboratory. Biochemically they may initially resemble *Salmonella* in many ways, such as hydrogen sulfide production and the inability to ferment lactose.

180.

B. The porphyrin test is commonly used to test for the X factor (hemin) requirement of *Haemophilus* spp. A positive test result indicates

that the organism possesses the enzymes to convert aminolevulinic acid (ALA) into porphyrins and, therefore, would not require hemin. If porphyrins are produced, this rapid test will show red fluorescence under UV light after a 4-hour incubation period.

181.

A. *Bordetella bronchiseptica* in humans produces either a respiratory illness or wound infections. The organism is a part of the normal respiratory flora of laboratory animals such as rabbits and guinea pigs. *B. bronchiseptica* may cause problems for researchers because it can cause outbreaks of bronchopneumonia in experimental animals. It also causes kennel cough in canines.

182.

B. *Escherichia coli* is frequently the etiologic agent of community-acquired cystitis. This agent can be easily recognized by its fermentation of lactose, negative citrate reaction, and positive indole test. On eosin methylene blue agar, *Escherichia coli* produces characteristic dark colonies with a metallic sheen.

183.

C. "Satellitism" is the name given to the appearance of colonies of *Haemophilus influenzae* on sheep blood agar medium around colonies of organisms that provide an essential growth factor. *H. influenzae* requires both hemin and NAD. Colonies of some organisms, such as *Staphylococcus* and *Neisseria*, produce NAD, which diffuses into the surrounding agar and enables *H. influenzae* to grow.

184.

A. *Bordetella pertussis* is the etiologic agent of pertussis, or whooping cough. On Bordet-Gengou or Regan-Lowe agars, the organism forms small, round colonies that resemble mercury droplets. A nasopharyngeal swab is recommended as the optimal specimen for the recovery of this agent.

185.

C. *Neisseria meningitidis* is the etiologic agent of one form of inflammation of the meninges, known as epidemic cerebrospinal meningitis. Infection with *Bordetella pertussis* produces the highly contagious upper respiratory infection pertussis. Both diseases are spread by droplet infection or fomites contaminated with respiratory secretions. The microorganisms are present in greatest numbers in the upper respiratory tract, and specimens for isolation and identification should be collected on nasopharyngeal swabs.

186.

C. *Salmonella* Typhi is commonly spread by chronic carriers. Without treatment, this enteric bacillus can be carried throughout a person's lifetime and is sequestered most often in the gallbladder. Carriers are usually asymptomatic, and the presence of the organism can be confirmed only by isolation and identification in the clinical laboratory.

187.

A. *Brucella* infects cattle and may be transmitted to humans by the ingestion of contaminated milk or other dairy products. Milk is able to support the growth of many clinically significant microorganisms, which may often be ingested in unpasteurized dairy products. Melioidosis and glanders are caused by *Burkholderia pseudomallei* and *B. mallei*, respectively. Pontiac fever is caused by *Legionella pneumoniae*. None of these is transmitted by milk.

188.

B. *Pseudomonas aeruginosa* is a major cause of hospital-acquired infections. These opportunistic organisms are able to survive in moist environments for prolonged periods and may be transferred to immunocompromised patients. *Pseudomonas* infections in recent years have accounted for as much as 10% of nosocomial infections.

189.

B. A variety of media has been developed to aid in the isolation of *Neisseria gonorrhoeae* from specimens containing mixed flora. Examples include Martin-Lewis, modified Thayer-Martin, GC-Lect, and New York City media. The most commonly used nonselective medium for the isolation of *N. gonorrhoeae* is chocolate agar. Cefsulodin-irgasan-novobiocin (CIN) is a selective and differential medium for the isolation of *Yersinia enterocolitica* and *Aeromonas*.

190.

A. Hektoen enteric agar was developed to improve the isolation of *Shigella* and *Salmonella* from stool specimens. The selective nature of this agar is due to bile salts. The medium also contains three carbohydrates—lactose, sucrose, and salicin—along with a pH indicator to detect carbohydrate fermentation. Fermentative organisms turn the medium yellow. Ferric ammonium citrate and sodium thiosulfate are included in the medium to detect H₂S production. H₂S-producing organisms appear as black-centered colonies.

191.

A. Thiosulfate-citrate-bile salt-sucrose (TCBS) agar is recommended for use in the selective isolation of *Vibrio* spp. associated with cholera, diarrhea, or food poisoning. The selective agent in this medium to inhibit gram-positive organisms is oxgall, a naturally occurring substance containing bile salts and sodium cholate. Sucrose is the carbohydrate in the medium. *V. cholerae* and *V. alginolyticus* ferment sucrose and appear as large yellow colonies. *V. parahemolyticus* is unable to ferment sucrose and exhibits colonies with blue to green centers.

192.

A. The oxidase test detects those organisms that produce the enzyme cytochrome oxidase. A 1% solution of dimethyl- or tetra-methyl-*p*-phenylenediamine dihydrochloride is applied to filter paper, and the test organism is then rubbed into the impregnated area. Because Nichrome wire may cause a false-positive result, a platinum or plastic loop or wooden applicator stick should be used to pick the colony. The rapid development of a dark purple color in the area where the organism was inoculated is a positive oxidase test.

193.

B. Strains of *Haemophilus* able to synthesize heme are identified by the porphyrin test. Species such as *H. influenzae*, which require heme, would give a negative test result, whereas *H. parainfluenzae* would be positive. A red color is indicative of a positive reaction in this test.

194.

B. Direct fluorescent antibody (DFA) test results for *Bordetella pertussis* are rapid but presumptive. Both positive and negative test results must be confirmed by culture. The quality of the test result depends greatly on the experience of the microscopist, the quality of the antibody, and the microscope. Material collected from the

nasopharynx with calcium alginate swabs for DFA and culture is recommended.

195.

C. Exotic pets such as iguanas, snakes, and turtles are known to carry *Salmonella*. Young children who do not practice good handwashing after touching family pets are particularly at risk for infection. Natural medicinal products made from snakes or other animals known to carry *Salmonella* have been implicated in cases of salmonellosis.

196.

B. Keratitis is a serious clinical condition that is characterized by inflammation of the cornea, which, if not appropriately treated, may lead to loss of vision. *Pseudomonas aeruginosa* is the most common agent of bacterial keratitis associated with lens-cleaning solution. Pseudomonads are opportunistic pathogens that are commonly associated with contaminated fluids.

197.

B. *Helicobacter pylori* is implicated as an etiologic agent of gastritis and peptic ulcer disease. This organism can be demonstrated in gastric biopsy specimens. *H. pylori* produces a strong positive urease test result.

198.

D. New York City (NYC) medium was developed by the New York City Public Health Laboratory for the isolation of *Neisseria gonorrhoeae*. It is a horse serum-based medium that is selective by the addition of colistin, vancomycin, and amphotericin B. Modified Thayer-Martin, another commonly used selective medium for *N. gonorrhoeae*, is chocolate based.

199.

B. Like *Pseudomonas aeruginosa*, *Burkholderia cepacia* is a ubiquitous opportunistic organism. Although *P. aeruginosa* is by far the most important cause of lower respiratory tract infections in patients with cystic fibrosis, *B. cepacia* is also a significant cause of morbidity. Both of these bacteria are oxidase positive and will grow on MacConkey agar. *P. aeruginosa* typically produces a green discoloration of the medium it is grown on.

200.

A. *Eikenella corrodens* can be normal flora of the oral cavity of humans. It is a weak pathogen that is associated with polymicrobial abscesses of the oral cavity. *E. corrodens* will grow on sheep blood and chocolate agars. Some strains will produce pitting of the agar.

Mycobacteria

201.

A. Mycobacteria characteristically possess a high lipid content, unlike gram-positive cocci and gram-negative bacteria. The high lipid content acts to protect these organisms from dehydration and the lethal effects of alkali, various germicides, alcohol, and acids. Thus, these bacteria do not stain well with the Gram stain, and an acid-fast staining technique must be used.

202.

D. The optimal growth temperature of *Mycobacterium xenopi* is 42°C, which enables its survival and replication as an environmental contaminant in hot water systems. Human infections caused by *M. xenopi* are rare. The majority of clinically significant *Mycobacterium* spp., those not known to cause cutaneous infections, have an optimal growth temperature of 37°C.

203.

B. Growth on MacConkey agar is a test used for differentiation of rapidly growing mycobacteria. The MacConkey agar used for mycobacteria identification is a different formulation than that used for enterics, in that crystal violet is omitted. A MacConkey agar plate is inoculated with a 7-day broth culture of the test organism. The inoculated plate is then incubated at 37°C. Plates are checked for growth at 5 days, and if no growth is detected, they are checked daily until day 11, at which time they are discarded as negative. *M. fortuitum* and *M. chelonae* are the only mycobacteria able to grow on MacConkey agar in 5 days.

204.

D. *Mycobacterium scrofulaceum* is defined as a scotochromogen because of its characteristic of producing pigmentation in the dark. This slowly growing *Mycobacterium* is a cause of cervical adenitis and other types of infections predominantly in children. Therapy may require susceptibility studies that include the secondary drugs, because the organism is known in some cases to be resistant to isoniazid and streptomycin.

205.

C. The *N*-acetyl-L-cysteine-sodium hydroxide (NALC-NaOH) method is recommended because the addition of NALC allows the concentration of NaOH to be reduced to 2%. The NALC is a mucolytic agent that frees trapped organisms in the sample, and the NaOH acts as a decontaminant. The optimal treatment reduces the numbers of indigenous microorganisms present in the sample without significantly reducing the number of tubercle bacilli.

206.

C. Hansen disease (leprosy) is caused by *Mycobacterium leprae*. Chronic skin lesions and sensory loss characterize this disease. Skin or biopsy specimens taken from within the margin of a lesion will demonstrate the causative agent. Cultures of this agent on artificial media, unlike other mycobacteria, have not been successful. Cultivation can be accomplished by injecting bacilli into the foot pads of mice or systemically into armadillos.

207.

C. The Centers for Disease Control and Prevention has adopted the diagnostic standards recommended by the American Thoracic Society as published in 1981. This is a method of reporting the number of acid-fast bacilli observed in fuchsin-stained smears of clinical material. Up to nine acid-fast bacilli per field should be reported as a positive, at 3+.

208.

C. Acid-fast bacilli can be demonstrated in stained smears of clinical material using the Ziehl-Neelsen or Kinyoun acid-fast stains. The Kinyoun carbol-fuchsin method uses a higher concentration of phenol in the primary stain to accelerate the staining process. Therefore, unlike the Ziehl-Neelsen stain, the Kinyoun stain does not need to be heated.

209.

C. Members of the genus *Mycobacterium* are characterized as obligate aerobic bacilli that, because of the high lipid content of their cell wall, exhibit acid fastness when stained. Most species pathogenic for humans are slowly growing. *Mycobacterium kansasii* is a slowly growing photochromogen.

210.

C. *Mycobacterium ulcerans* and *Mycobacterium marinum* have both been implicated in skin infections. Their predilection for surface areas of the body is related to their optimal growth temperature range of 30–32°C. At body temperature (37°C) or higher, these organisms grow poorly, if at all.

211.

B. Rapid development of drug resistance is a concern in the treatment of tuberculosis. Patients are treated generally with a combination of at least two of the primary drugs, such as isoniazid, rifampin, ethambutol, and pyrazinamide. Because of the slowly growing nature of the bacteria, they are innately resistant to a number of agents.

212.

B. NALC (*N*-acetyl-L-cysteine) is a mucolytic agent used in decontamination and digestion procedures for the recovery of mycobacteria. NALC liquefies mucus, releasing trapped bacteria. NaOH, between 2 and 4%, is frequently used as a bactericidal agent to prevent the overgrowth of normal flora in clinical specimens.

213.

D. A positive tuberculin skin test reaction is an example of a hypersensitivity reaction. Tuberculin preparations are prepared from culture filtrates, which are precipitated with trichloroacetic acid and are known as purified protein derivative (PPD). A positive test demonstrates an area of induration following an intradermal injection of PPD.

214.

A. *Mycobacterium bovis* causes tuberculosis in cattle. This agent is an etiologic agent of tuberculosis in humans as well, and it must be differentiated from *M. tuberculosis* when recovered from clinical material. Unlike *M. tuberculosis*, *M. bovis* is negative for niacin production and nitrate reduction.

215.

A. The *M. avium* complex is sometimes referred to as *Mycobacterium avium-intracellulare* complex. These slowly growing bacilli are uncommon in immunocompetent individuals. These bacteria cause disseminated infections in patients with acquired immunodeficiency syndrome and are important causes of morbidity and mortality in these patients.

216.

B. *Mycobacterium gordonae* has been recovered from water stills, faucets, and bodies of water in nature, which is why it has been called the “tap water scotochromogen.” These organisms are not considered to be pathogenic for humans, but because they may be recovered as contaminants, their identification is recommended. Members of Runyon group II, they are slow growing and form yellow-orange colonies that do not depend on exposure to light.

217.

C. The human tubercle bacillus is *Mycobacterium tuberculosis*. Growth of this well-known human pathogen appears in 2 to 3 weeks when incubated at 35°C. These niacin-positive mycobacteria form dry heaping colonies that are buff colored.

218.

C. *Mycobacterium marinum* is the causative agent of “swimming pool granuloma.” Typically,

patients with abraded skin come in contact with water containing this agent and develop granulomatous skin lesions. Lesions generally occur on the extremities, because the skin temperature is close to the organism’s optimal growth temperature of 25–32°C.

219.

B. *Mycobacterium bovis* is susceptible to 5 µg/mL of thiophene-2-carboxylic acid hydrazide (T2H). This *Mycobacterium* is associated with cattle and is rarely isolated from humans in the U.S. Growth occurs only at 35°C and is differentiated from other mycobacteria by its susceptibility to T2H.

220.

A. Auromine-rhodamine is a fluorescent stain used to visualize the mycobacteria. The bacteria retain the stain and will appear bright yellow against a black background. Because it is easier to see the bacilli, this stain is more sensitive than a fuschin-based stain (e.g., Ziehl-Neelsen). The calcofluor white stain is a fluorescent stain used to visualize fungi.

221.

B. *M. kansasii* is the most commonly isolated photochromogen in the U.S. It is the second most commonly isolated nontuberculosis *Mycobacterium* sp. behind *M. avium* complex. *M. kansasii* produces chronic lung disease resembling classic tuberculosis.

222.

B. Skin cultures for the recovery of *Mycobacterium* spp. should be incubated at 30°C. The mycobacteria associated with these type of infections include *M. ulcerans*, *M. marinum*, and *M. haemophilum*. The optimal temperature for these slow growers is 30°C.

223.

D. Lowenstein-Jensen, Lowenstein-Jensen-Gruft, and Middlebrook media are commonly used for the isolation of the mycobacteria. Chocolate agar will support the growth of *Mycobacterium haemophilium*; however, chocolate agar is not routinely used for cultures of mycobacteria. This species requires ferric ammonium citrate or hemin for growth and also has an optimal temperature of 30°C.

224.

A. The mycobacteria are only slightly more resistant to the decontamination procedures than other bacteria. Therefore, it is only appropriate to decontaminate specimens for mycobacteria that are contaminated with normal flora. Because sputum passes through the oral cavity, it contains a large amount of normal oral flora. The other specimens listed are typically sterile and lack normal flora.

225.

C. *Mycobacterium leprae* is the causative agent of Hansen disease (leprosy). This bacterium cannot be grown on artificial media and requires laboratory animals for cultivation. The optimal temperature for *M. leprae* is lower than the core body temperature of 37°C; therefore, infections generally occur in the skin in the extremities. The bacteria are likely spread from nasal secretions and not the lesions; they are not highly contagious, as most people believe.

Anaerobic Bacteria

226.

B. Improperly home-canned foods, especially low-acid-content vegetables, cause the majority of the cases of food-borne botulism. The ubiquitous nature of *Clostridium botulinum* enables the spores to contaminate a variety of foods. Contamination and subsequent germination

under anaerobic conditions stimulate toxin formation. The patient becomes ill following the ingestion of food that contains nanograms of preformed toxin.

227.

B. Zoonotic diseases are diseases of animals that are transmissible to humans. Leptospirosis is primarily a disease of small animals such as rabbits. It is contracted by humans through contact with infected carcasses or contaminated water. *Bacillus anthracis* is found in the environment. Anthrax is transmitted to humans by exposure to contaminated animal products such as cattle hides, goat hair, or wool. Brucellosis is associated with a variety of animals.

228.

A. Isolates of the anaerobic, spore-forming bacillus *Clostridium perfringens* characteristically produce a pattern of double zone hemolysis on sheep blood agar plates. A Gram stain of such colonies should demonstrate a medium-sized gram-positive bacillus that does not contain spores. For further identification the isolate should be inoculated on an egg yolk agar plate to detect lecithinase production.

229.

D. *Prevotella melaninogenica* was isolated from this cervical abscess. This anaerobic organism is part of the indigenous microflora of the respiratory, gastrointestinal, and genitourinary tracts and is considered a significant human pathogen. The black pigment appears after several days when growing on laked blood agar plates. Prior to pigmentation, this isolate can be presumptively identified by its brick-red fluorescence under UV light. Pigmented *Porphyromonas* spp. are asaccharolytic.

230.

A. *Clostridium tetani* is an obligate anaerobe. Spores are widespread in nature and cause disease by contaminating puncture wounds. The exotoxin, tetanospasmin, produced by this organism is one of the most powerful bacterial toxins known.

231.

B. The gram-positive, non-spore-forming, anaerobic bacillus *Actinomyces israelii* is a slowly growing organism that is considered to be an opportunistic pathogen. Colonies may not be visible before 5 to 7 days or longer. When colonies are seen, they appear white, opaque, lobate, irregular, and shiny and are described as resembling a molar tooth. *A. israelii* is part of the indigenous flora of the human mouth, and a few *Actinomyces* spp. have been found to inhabit the vagina. Pathogenesis generally involves trauma to tissues of a mucous membrane and the introduction of this endogenous organism.

232.

A. The predominant indigenous flora of the human intestinal tract is anaerobic, gram-negative, non-spore-forming bacilli. The *Bacteroides fragilis* group, in particular, predominates in the fecal flora. Trauma involving the intestinal area or bowel surgery predisposes patients to an endogenous anaerobic infection. Although these organisms are present in large numbers, their routine identification in fecal cultures is of no diagnostic value.

233.

A. Members of the *Bacteroides fragilis* group, the most commonly isolated anaerobes and a predominant part of the indigenous fecal flora in humans, are not inhibited by the presence of bile. Bile-esculin agar plates are used for the selection and presumptive identification of the *B. fragilis* group. Although not used as a

component of selection media for the *Bacteroides* group, it is important to note that, in general, gram-negative, non-spore-forming, anaerobic bacilli are susceptible to penicillin. The *B. fragilis* group is an exception in that it is known to be resistant to penicillin.

234.

B. The second most commonly encountered group of anaerobes in human infections is the anaerobic, gram-positive cocci. They may account for one-fourth of all anaerobes isolated in clinical laboratories. Estimating their clinical significance, however, is often difficult. Important isolates include *Fingoldia magna* (formerly *Peptostreptococcus magnus*) and *Peptostreptococcus anaerobius*.

235.

A. The closed chest abscess described is characteristic of human actinomycosis, which is caused by *Actinomyces israelii*, an anaerobic, gram-positive, non-spore-forming bacillus. The organism is not acid-fast, which helps to differentiate it from *Nocardia* spp. Actinomycotic pus characteristically shows "sulfur granules" or solid yellow particles made up of masses of the filamentous bacilli seen on the Gram stain in Color Plate 29■.

236.

A. Kanamycin-vancomycin laked blood (KVLB) agar is selective for the *Prevotella* and *Bacteroides* spp. Presumptive identification of *B. fragilis* group can be accomplished utilizing its antimicrobial resistance pattern. *Bacteroides* spp. are resistant to vancomycin and kanamycin, unlike *Fusobacterium* spp., which are resistant to vancomycin but susceptible to kanamycin. A KVLB agar plate should be part of the primary plating media for anaerobic cultures.

237.

C. *C. difficile* is an important cause of a hospital-acquired infection commonly called pseudomembranous colitis. Hospitalized patients treated with broad-spectrum antimicrobial agents become colonized when their normal intestinal flora is diminished. The most rapid and accurate diagnostic method is detecting toxins A and/or B in stool specimens. Cycloserine-cefoxitin-fructose agar (CCFA) is the recommended selective medium for *C. difficile*, although few laboratories attempt isolation.

238.

D. Although it has a gram-positive-like cell wall, *Mobiluncus* stains gram-variable to gram-negative. This curved and motile bacillus seems to contribute to the pathology of bacterial vaginosis (BV). A Gram stain of the discharge that is produced in this condition can be used for the detection of these distinctively curved organisms. The presence of “clue cells,” gram-variable pleomorphic bacilli on vaginal epithelial cells, is diagnostic of BV.

239.

B. Infant botulism or “floppy infant” syndrome is seen in children up to 6 months of age. This infectious process begins with the ingestion of food contaminated with spores of *Clostridium botulinum*. Following ingestion, viable spores are carried to the lower bowel, where they germinate and elaborate the powerful neurotoxin that produces the characteristic flaccid paralysis.

240.

D. *Propionibacterium acnes* is the most frequently isolated of all the gram-positive, non-spore-forming, anaerobic bacilli. It is a part of the normal human bacterial flora and predominates on the surface of the body, but may also be recovered from the upper respiratory tract, intestines, and urogenital tract. This organism is a

common contaminant of blood cultures because of its presence on the skin. Care in the preparation of the skin before venipuncture helps to eliminate confusion caused by the recovery of this anaerobic isolate.

241.

D. The spore of *Clostridium tetani* is located terminally and is larger than the sporangium. Characteristically, when seen on Gram stain, the cells of *Clostridium tetani* resemble a drumstick or tennis racket. Spores can be readily seen in late growth phase cultures incubated at 37°C.

242.

C. *Fusobacterium nucleatum*, a gram-negative, anaerobic bacillus, is part of the indigenous microbial flora of the respiratory, gastrointestinal, and genitourinary tracts. It is frequently implicated as the causative agent in metastatic suppurative infections such as brain abscesses. These pale-staining bacilli characteristically appear as long, thin bacilli with pointed ends.

243.

C. *Propionibacterium* spp. are non-spore-forming, anaerobic, gram-positive bacilli. *Clostridium* spp. typically form spores, although it is difficult to induce some species to form spores *in vitro*. *Veillonella* is a gram-negative coccus, and *Fusobacterium* is a gram-negative bacillus.

244.

C. *Clostridium botulinum* is the causative agent of botulism, a disease produced by an exotoxin that acts on the central nervous system. Types A, B, E, and F are causes of human botulism; types C and D and less commonly types A and B are associated with disease in animals and birds. Type G has not been associated with disease in humans or animals. This anaerobic organism produces oval, central, or subterminal, spores that germinate in food products or less commonly in wounds.

245.

D. Resazurin is an E_h indicator used in anaerobic culture media. When the oxygen concentration is reduced, the resazurin indicator is colorless. A pink color in the medium indicates aeration and an unsuitable environment for the preservation of obligate anaerobic organisms.

246.

B. *Fusobacterium nucleatum* is the most frequent clinical isolate within the genus *Fusobacterium*. These anaerobes are part of the indigenous flora of human mucous membranes, oral cavity, intestine, and urogenital tract. *F. necrophorum* is, however, much more virulent.

247.

B. *Clostridium septicum* is isolated in the clinical laboratory in cases of serious or often fatal infections. Bacteremia is seen in association with an underlying malignancy. The most common types of cancer are colon or cecum, breast, and leukemia or lymphoma.

248.

A. Botulinal toxin is the most potent exotoxin known. When absorbed, this exotoxin produces the paralyzing disease botulism. Toxin acts in the body by blocking the release of acetylcholine in the neuromuscular junction of the peripheral nervous system, causing muscle paralysis.

249.

B. *Clostridium perfringens* is the species most commonly associated with clostridial myonecrosis or gas gangrene. These soil and water saprophytes most frequently gain entrance to the human body through traumatic wounds. Once they have been introduced into injured tissue, the characteristic syndrome of myonecrosis due to the elaboration of exotoxins may occur. Other species involved with myonecrosis are

C. septicum, *C. novyi*, *C. sordellii*, and *C. histolyticum*.

250.

D. *Eubacterium*, *Bifidobacterium*, and *Propionibacterium* are all anaerobic, gram-positive, non-spore-forming bacilli. This group of anaerobic microorganisms is difficult to identify in the clinical laboratory and often requires the use of gas chromatography. These organisms are rarely isolated. *Suttonella wadsworthensis* is an anaerobic, gram-negative bacillus.

251.

D. *Clostridium tetani* is an obligate anaerobe. Spores are widespread in the soil. When introduced into a puncture wound, the spores require the reduced oxygen environment produced by the necrotic tissue and poor blood supply in the wound. Cleaning and debridement of the wound is important, as is the administration of a tetanus toxoid booster. Many clostridia require anaerobic conditions for spore formation.

252.

B. Most *Clostridium* spp. are gram-positive, and they generally form spores. Because they are obligate anaerobes, they will not grow on sheep blood or chocolate agars incubated aerobically. *Bacillus* spp. also form spores, but they are facultative anaerobes and would therefore grow on media incubated aerobically. *Lactobacillus* is a non-spore-forming, gram-positive bacillus, and *Prevotella* is a gram-negative bacillus.

253.

C. *Lactobacillus* spp. are normal flora of the vagina and digestive tract and are rarely pathogenic. They are aerotolerant anaerobes and will produce alpha-hemolysis on sheep blood agar plates incubated aerobically. These organisms can also produce a green discoloration on chocolate agar.

254.

C. Clostridium perfringens produces spores that are oval and central in location but that are rarely seen in foods or on laboratory cultures. This organism is divided into five types, A to E, based on the quantities and types of exotoxins produced. Type A is responsible for human cases of myonecrosis and food poisoning. Alpha-toxin or lecithinase is produced by all strains of *C. perfringens*.

255.

D. Clostridium tetani is a strict anaerobe that is motile and produces terminal round spores. Biochemically it does not utilize carbohydrates, with the rare exception of glucose. *C. tetani* is gelatinase and indole positive but is nonproteolytic and H₂S negative. The clinical manifestations of tetanus are the result of the release of a neurotoxic exotoxin.

256.

C. Fusobacterium nucleatum characteristically appears on Gram stain as a gram-negative rod with pointed ends. Its growth is inhibited by a 1-μg kanamycin disk and the presence of bile. The *Bacteroides fragilis* group and the pigmented species *Prevotella* and *Porphyromonas* are not inhibited by kanamycin.

257.

D. Prevotella melaninogenica can be rapidly presumptively identified on media containing laked blood with the use of an ultraviolet light source. This important anaerobic pathogen can be differentiated after 5 to 7 days' incubation by its black pigmentation. The use of ultraviolet light enables a more rapid differentiation because of the appearance of a brick red fluorescence before the pigment is demonstrated.

258.

C. The symptoms of *Clostridium difficile* infection are toxin mediated. This organism is known to cause pseudomembranous colitis associated with the use of antimicrobial therapy. Cytotoxins can be directly detected in stools by enzyme immunoassays. These tests have generally replaced the use of cell culture monolayers, which were examined after incubation for evidence of cytotoxicity (i.e., cytopathic effect).

259.

B. A reverse CAMP test aids in the identification of *Clostridium perfringens*. In this test, a single straight streak of *Streptococcus agalactiae* is made down the center of the plate. Suspected *C. perfringens* isolates are inoculated at right angles to the *S. agalactiae* inoculum. After anaerobic incubation, *C. perfringens* will exhibit enhanced hemolysis at the intersection where the two species meet.

260.

A. The identification of *Peptostreptococcus anaerobius* is made easier by the use of the sodium polyethanol sulfonate (SPS) disk. The test is performed by growing the organism in the presence of a disk impregnated with SPS. A zone of inhibition of 12–18 mm around the disk is considered sensitive and a presumptive identification of this organism.

261.

C. *Fusobacterium nucleatum* is a thin gram-negative rod with pointed ends and a slightly curved appearance in fresh isolates. As the bacteria are subcultured, they may lose their curved appearance and appear as thin rods. *F. nucleatum* is found in human specimens and is considered clinically significant.

262.

D. Anaerobes are a major cause of brain abscess. *Peptostreptococcus* spp. are associated with human disease, usually in polymicrobial infections, and can be seen on a Gram stain of clinical material. The characteristic Gram stain morphology of *Fusobacterium* would enable a physician to make a presumptive identification of the presence of anaerobic flora in this clinical case.

263.

C. *Bacteroides fragilis* stimulates abscess formation. The capsule is a contributing factor to the pathology produced by this anaerobe. *B. fragilis* is the most common anaerobic gram-negative bacillus isolated in the clinical laboratory.

264.

D. The *Bacteroides fragilis* group is among the most antimicrobial-resistant anaerobes. Beta-lactamase production is responsible for their resistance to the penicillins. These anaerobes are also resistant to first-generation cephalosporins and aminoglycosides.

265.

B. The common agents in cases of aspiration pneumonia are oral anaerobes, such as the black-pigmented *Prevotella* and *Porphyromonas*, and *Bacteroides*, fusobacteria, and anaerobic streptococci. These endogenous organisms, when in an abnormal site, possess virulence factors that enable them to produce disease. Often these are polymicrobial infections mixing anaerobes with aerobic or facultative organisms such as *Enterobacteriaceae* or *Staphylococcus aureus*. *Mobiluncus* is not associated with aspiration pneumonia.

266.

B. Methylene blue strips are the most commonly used oxidation-reduction (E_h) indicators. When anaerobic conditions are achieved, the methylene

blue indicator will turn from blue (oxidized) to white, indicating reduction. Resazurin, another E_h indicator, is used in anaerobic transport systems and anaerobic culture media such as the pre-reduced anaerobically sterilized (PRAS) system. Resazurin when oxidized is pink; when reduced, the color fades to white, indicating anaerobiosis.

267.

A. An area of precipitate in the agar around the colonies indicates that the organism produced lecithinase. Lecithinase (alpha-toxin) cleaves lecithin in the medium, producing an insoluble product. *Clostridium perfringens* is positive for lecithinase.

268.

B. *Peptostreptococcus niger* produces a pigment that begins olive-green and gradually becomes black. This is the only species in the genus. It is a weak pathogen sometimes found in polymicrobial infections.

269.

C. *Clostridium perfringens* is one of the most important causes of food-borne diseases in the U.S. The bacterial spores can survive cooking (typically found in meats and gravies), and upon cooling they germinate into vegetative cells. When the bacteria are ingested, they sporulate in the intestinal tract. The enterotoxin is a spore coat protein made in excess and released by the bacteria.

270.

C. *Lactobacillus* spp. are found as normal flora in the gastrointestinal and female genital tract. The bacteria produce acids from the metabolism of carbohydrates, resulting in an acid environment in the vagina. If the population of lactobacilli decreases, the vaginal pH will rise toward neutrality. This favors the growth of other bacteria, such as *Mobiluncus*, that can result in bacterial vaginosis.

Chlamydia, Rickettsia, and Mycoplasma

271.

B. *Chlamydia trachomatis* is the causative agent of inclusion conjunctivitis, trachoma, and genital tract infections, including lymphogranuloma venereum. Trachoma is a primary cause of blindness worldwide. The disease is preventable, but when it is not treated, the organism produces hypertrophy of the lymphoid follicles on the inner surface of the upper eyelid. This process causes the upper eyelid to evert (entropion), which ultimately leads to blindness.

272.

A. The *Chlamydia* and *Chlamydiophila* are obligate intracellular parasites. They require ATP from their host cell. As such, these bacteria cannot be grown on artificial media. They can be cultivated in cell cultures.

273.

C. *Mycoplasma pneumoniae* causes primary atypical pneumonia. The pneumonia is atypical in that it is milder than the pneumonia caused by *Streptococcus pneumoniae*. Chest X-rays of patients with atypical pneumonia may show bilateral infiltrates, although physical examination reveals few chest findings.

274.

D. Provided that arginine is added for *Mycoplasma hominis*, SP4 agar or broth can be used for the growth of *M. pneumoniae* and *M. hominis*. *M. pneumoniae* is a slow-grower, so most infections are diagnosed by serologic assays. *M. pneumoniae* is an important respiratory tract pathogen of humans. It is found only in humans and is typically spread person to person.

275.

C. Human infections with *Chlamydiophila psittaci* (psittacosis) occur after exposure to infected birds and their droppings. A true zoonosis, psittacosis is a disease of birds that may be contracted by humans. The disease produced by this organism may be mild or fulminant, the latter of which has a high mortality rate. Clinical manifestations of the disease include severe headache, weakness, and mild pulmonary symptoms.

276.

C. Unlike rickettsial diseases, no rash occurs in *Coxiella burnetti* infections. The organism is an obligate intracellular parasite that is able to survive for long periods in the environment. It causes a zoonosis and is transmitted to humans by inhalation and contact with fomites. Infections can also be acquired by ingestion of unpasteurized milk.

277.

A. Mycoplasmas are small, pleomorphic organisms that lack a cell wall and are best visualized by darkfield or phase microscopy. Penicillin is not an effective treatment because of their lack of a cell wall, and isolation requires media supplemented with peptone, yeast extract, and serum. Species of the genus *Mycoplasma* are well-known human pathogens that cause a variety of disease processes.

278.

A. *Chlamydia trachomatis*, a leading cause of blindness, can be detected in corneal scrapings of suspected cases of trachoma and inclusion conjunctivitis. Clinical material can be examined directly using fluorescent antibody techniques or can be cultured on McCoy cells. Trachoma is a chronic inflammatory process of the conjunctiva that results in corneal involvement.

279.

C. *Mycoplasma hominis*, *M. genitalium*, and *Ureaplasma urealyticum* have been linked to human genital infections. These species can also be isolated from asymptomatic sexually active adults. *M. pneumoniae* is primarily a respiratory tract pathogen.

280.

B. *Chlamydia pneumoniae* is an important cause of sporadic and epidemic lower respiratory tract disease characterized as atypical pneumonia. The organism is a human pathogen spread person to person. Most infections are diagnosed serologically. Tetracycline and erythromycin are effective treatments. The organism has been associated epidemiologically to coronary heart disease.

281.

C. Polymerase chain reaction DNA amplification has been shown to be more sensitive than cell culture and nearly 100% specific for the detection of *Chlamydia trachomatis*. Suitable specimens for detection are cervical secretions and urine. When confirmation of *C. trachomatis* is needed, tissue culture remains the method of choice.

282.

C. *Mycoplasmas* are implicated in a variety of human infections. *M. pneumoniae*, in particular, is

a clinically important respiratory tract pathogen. When grown on culture media, colonies, most notably *M. hominis*, are said to have a “fried egg” appearance because the central portion of the colony has grown into the agar and thus appears more dense and is slightly raised.

283.

A. Q fever is caused by infection with *Coxiella burnetii*, which has unique characteristics. Unlike other rickettsiae, this organism is able to resist heat and drying for long periods and does not rely on an arthropod vector for transmission. Infectious fomites such as dust from contaminated cattle hides and fluids released during birth are considered the primary modes of infection.

284.

A. Direct microscopic examination for *Rickettsia* organisms is possible using such stains as Giemsa, Machiavello, or Gimenez. The recommended procedure is the nonspecific Gimenez stain, which colors the organisms a brilliant red against a green background. The staining technique calls for flooding a thin smear, which has been air dried, with a solution of carbol-fuchsin for 1–2 minutes. After washing with tap water, malachite green is added for 6–9 seconds before the final washing with tap water.

285.

D. Transovarian passage from generation to generation in ticks perpetuates *Rickettsia rickettsii* for several generations outside an animal host. A blood meal serves to reactivate the rickettsiae carried by the arthropod vector. Rodents and small mammals are the natural reservoirs for the rickettsiae that cause this form of spotted fever.

286.

D. Flying squirrels, *Glaucomys volans*, are associated with cases of the sylvatic form of typhus in the U.S. The squirrel louse transmits the organism among the squirrel population. Humans contract the disease through association with infected squirrels. The disease is more common in the winter months, when squirrels seeking shelter enter dwellings.

287.

A. Humans who have had the classic form of typhus may remain infected with the causative agent *Rickettsia prowazekii*. Relapses or recrudescence of disease may occur in these persons years or decades after the initial attack. The latent form of infection is known as Brill-Zinsser disease and may serve as an interepidemic reservoir for epidemic typhus.

288.

D. Murine typhus is transmitted to humans by fleas infected with *Rickettsia typhi*. Prevalent in the southern U.S., it is primarily a disease of rodents and is sometimes transmitted to humans. Control of disease outbreaks is related to rodent (rat) control and the related rat flea population. The symptoms of murine or endemic typhus are similar to those of the classic epidemic form seen in Europe.

289.

C. *Mycoplasma pneumoniae* produces hydrogen peroxide, which causes lysis of red blood cells. Suspected *M. pneumoniae* growing on SP4 agar can be overlaid with a molten agar containing guinea pig red blood cells. After incubation, a small zone of beta-hemolysis will be seen around the colonies.

290.

D. 10 B broth is used with genital specimens to isolate *Ureaplasma urealyticum*. The bacterium

requires urea and produces a strong alkaline pH because of the activity of urease. The bacteria are slow growers and form tiny colonies. The broth will typically not appear turbid.

Spirochetes

291.

D. Cardiolipin is a tissue lipid produced as a byproduct of treponemal infection. Nontreponemal tests for syphilis take advantage of antibodies made to cardiolipin. The most commonly used tests are the rapid plasma reagin (RPR) for serum and the Venereal Disease Research Laboratory (VDRL) for cerebrospinal fluid.

292.

A. *Leptospira* spp. are most reliably detected during the first week of illness by the direct culturing of a blood sample. The media of choice are Fletcher semisolid and Stuart liquid medium, both of which are supplemented with rabbit serum. One or two drops of the patient's blood are added to 5 mL of culture medium, which is incubated in the dark at 30°C or room temperature for up to 6 weeks. After the first week of disease and lasting for several months, the urine becomes the specimen of choice for isolation of the organism. Direct microscopic examination is not reliable for detection because of the low numbers of organisms normally present in body fluids.

293.

C. Syphilis is caused by *Treponema pallidum* subsp. *pallidum*. Congenital syphilis occurs when a pregnant woman has a septicemia, and the spirochetes cross the placenta and infect the fetus. Infection can affect fetal development and cause premature birth or fetal death, or the pregnancy may go to term. Following *in utero* infection, the infant is most often born with lesions characteristic of secondary syphilis; perinatal death is not an uncommon consequence of infection.

294.

C. The description given is characteristic of members of the genus *Leptospira*. Blood and other fluids, such as cerebrospinal fluid and urine, are examined by direct darkfield microscopy and stained preparations for the presence of these organisms in suspected cases of leptospirosis. The number of organisms present in clinical samples is low, and detection is difficult even when concentration methods are used. Cultural and serologic tests are available for the diagnosis of disease produced by these organisms.

295.

B. The human body louse, *Pediculus humanus*, is the vector for *Borrelia recurrentis*. Pathogenic species not only have specific vectors but also well-defined geographical distributions. Epidemic relapsing fever is found in Ethiopia, Sudan, and parts of South America.

296.

C. The antigen in the VDRL test is cardiolipin. In this flocculation test, reagin, an antibody-like protein, is produced by infected patients. Reagin binds to cardiolipin-lecithin-coated cholesterol particles, causing the particles to flocculate and indicating a positive test result. The test must be read microscopically.

297.

A. Lyme disease was first described in 1975 following an outbreak in Lyme, Connecticut. The etiologic agent, *Borrelia burgdorferi*, is transmitted to humans by the tick vector *Ixodes dammini*. Clinically the disease peaks in the summer and produces an epidemic inflammatory condition characterized by skin lesions, erythema, headache, myalgia, malaise, and lymphadenitis. Rat-bite fever is caused by *Spirillum minus*. Relapsing fever is caused by *Borrelia*, and Q fever is caused by *Coxiella burnetii*.

298.

B. The basic structure of spirochetes is an outer membrane, cytoplasmic membrane-peptidoglycan complex, cytoplasm, and axial fibrils. The fibrils are attached to the cytoplasmic membrane close to the ends of the cell, extending along the body under the outer membrane. The axial fibrils most closely resemble bacterial flagella and are associated with motility of the organism.

299.

B. Spirochetes are gram-negative, but most do not stain with the Gram stain. Silver impregnation can be used to visualize them in smears. The direct observation using darkfield or phase microscopy is recommended to view these delicate, coiled cells in body fluids or tissue sections.

300.

C. Infections other than syphilis can cause a positive VDRL result. The VDRL test detects an antibody that is not directed against *T. pallidum* antigens. It is a good screening test for syphilis, but it is not highly specific. Confirmation with a specific treponemal test, such as the fluorescent treponemal antibody-absorbed (FTA-ABS) assay, is required.

Antimicrobial Agents and Antimicrobial Susceptibility Testing

301.

A. Standardization of the susceptibility testing procedure is essential for determining the susceptibility of an organism to antimicrobial agents. A #0.5 McFarland standard is used when adjusting the turbidity of the suspension of test organism. A #0.5 McFarland standard has a turbidity consistent with approximately 1.5×10^8 organisms/mL of broth or saline.

302.

D. Hemophilus Test Medium is recommended for use in the disk-agar diffusion susceptibility testing procedure of *Haemophilus*. The testing of *Haemophilus* spp. requires supplemented media to support the growth of these fastidious organisms. *In vitro* growth of *H. influenzae* requires the presence of accessory growth factors: X factor (hemin) and V factor (NAD).

303.

D. Sulfonamides act to interfere with the ability of bacteria to use *p*-aminobenzoic acid, which is a part of the folic acid molecule, by competitive inhibition. These chemotherapeutic agents are bacteriostatic and not bactericidal. The drug sulfisoxazole is a member of this group and is used in the treatment of urinary tract infections, especially those caused by *Escherichia coli*, which must synthesize folic acid for growth.

304.

D. Antimicrobial susceptibility testing is not routinely performed on all bacteria. Certain organisms are predictably susceptible to a variety of antimicrobial agents. Therefore, testing is not usually performed even when these organisms are the etiologic agents of infection. Bacteria for which susceptibility tests are usually not performed include *Streptococcus pyogenes* (group A *Streptococcus*), *Streptococcus agalactiae* (group B *Streptococcus*), and *Neisseria meningitidis*.

305.

C. Inhibitors of peptidoglycan synthesis such as penicillin act to inhibit cell wall development. Bacteria unable to produce peptidoglycan for their cell walls are subject to the effects of varying osmotic pressures. The peptidoglycan component of the cell wall protects the bacterium from lysis.

306.

C. The requirement of 99.9% killing defines the minimum bactericidal concentration (MBC) of an antimicrobial agent. The MBC test is an additional quantitative assessment of the killing effect of a drug on a specific patient isolate. This test, done to evaluate a drug's activity, is sometimes requested in cases of life-threatening infections.

307.

B. Even though clindamycin and erythromycin are in different classes, the mechanisms of resistance are similar. The presence of erythromycin can induce clindamycin resistance. The D-zone test is used to detect the presence of this inducible resistance.

308.

D. The therapeutic effect of antimicrobial therapy is often increased by the use of a combination of drugs. A combination of antimicrobials is said to be synergistic when the sum of their effects is greater than that derived from either drug when tested independently. A tenfold decrease in the number of viable cells from that obtained by the most effective drug in the combination is the definition of synergism. Synergistic combinations of antimicrobials are used primarily in the treatment of tuberculosis, enterococcal endocarditis, and certain gram-negative bacillus infections.

309.

C. Beta-lactamase production by strains of *Haemophilus influenzae* renders them resistant to the antibacterial effect of penicillin and ampicillin. It is recommended that rapid beta-lactamase testing be performed on isolates in life-threatening clinical infections such as meningitis. The rapid tests all rely on this enzyme's ability to act on a beta-lactamase ring and in turn produce a color change, which denotes a positive result due to the production of penicilloic acid.

310.

C. The recommended plating medium for use in both the disk diffusion and tube dilution susceptibility test procedures is Mueller-Hinton. Low in tetracycline and sulfonamide inhibitors, this medium has been found to show only slight batch-to-batch variability. For the susceptibility testing of fastidious organisms (e.g., *Streptococcus pneumoniae*), 5% lysed sheep blood may be added.

311.

B. The Kirby-Bauer or disk-agar diffusion susceptibility test requires that the pH of the agar be tested at room temperature to ensure an optimal range of 7.2–7.4 before use in the procedure. A sample of the Mueller-Hinton medium can be tested by macerating it in distilled water and testing with a pH meter electrode; a surface electrode is acceptable for direct testing. Another acceptable method is to allow the agar to solidify around the electrode of a pH meter and then obtain a reading.

312.

B. Metronidazole, a drug recommended for the treatment of amebic dysentery and trichomoniasis, is a synthetic compound that acts by inhibiting DNA synthesis. The use of this drug for treating anaerobic infections has gained emphasis in light of resistance patterns of many of the commonly recovered anaerobes. Metronidazole is consistently active against all gram-negative, anaerobic bacilli; is able to cross the blood-brain barrier; and is the only agent consistently bactericidal against susceptible isolates.

313.

D. Tobramycin, an aminoglycoside, is the only antibiotic, of those listed, that is bactericidal. Bactericidal antibiotics actually destroy the bacteria, whereas bacteriostatic drugs only arrest

the growth of the microorganism. All aminoglycosides, with the exception of spectinomycin, are bactericidal in their activity.

314.

B. The extended spectrum beta-lactamases (ESBLs) confer resistance to the extended spectrum cephalosporins such as ceftriaxone and cefotaxime. ESBLs cleave the antibiotic, inactivating it. So far, ESBLs have only been found in gram-negative bacteria.

315.

C. Most fastidious bacteria do not grow satisfactorily in standard *in vitro* susceptibility test systems that use unsupplemented media. For certain species, such as *Haemophilus influenzae*, *Neisseria gonorrhoeae*, *Streptococcus pneumoniae*, and other *Streptococcus* species, modifications have been made to the standard Clinical and Laboratory Standards Institute (CLSI) methods. In the case of *S. pneumoniae*, current CLSI broth dilution test conditions include cation-supplemented Mueller-Hinton broth with 5% lysed horse blood.

316.

B. *Haemophilus influenzae* should be tested for beta-lactamase production. The test can be performed directly, and the methods are rapid and reliable for the detection of penicillin and ampicillin resistance. Rapid test methods, in general, rely on a color change to detect the presence of this enzyme. A pH indicator may be used to detect the penicilloic acid produced when the beta-lactam ring of penicillin is cleaved, or a color change can be observed when the beta-lactam ring of a chromogenic cephalosporin is hydrolyzed by the enzyme.

317.

D. Tolerance is described as the ability of certain strains of organisms to resist lethal concentrations of antimicrobial agents like penicillin. The growth of these organisms is only inhibited by these cidal drugs. This mechanism of bacterial resistance is attributed to a deficiency of cell wall autolysins.

318.

C. The rise in antimicrobial-resistant isolates of *Mycobacteria tuberculosis* has been an important public health crisis. The accepted methods for determining the *in vitro* antimicrobial susceptibility of mycobacteria are based on the growth of the microorganisms on solid or in liquid media containing a specified concentration of a single drug. Two such methods that have been described and are in common use in the U.S. are the agar proportion method and the BACTEC 460TB radiometric method.

319.

B. Clavulanic acid is a beta-lactamase inhibitor. It can be administered with amoxicillin or ticarcillin and is effective in treating infections caused by beta-lactamase-producing bacteria such as staphylococci, *Klebsiella*, and *Haemophilus influenzae*. Sulbactam and tazobactam are also beta-lactamase inhibitors.

320.

D. Vancomycin, which acts to inhibit cell wall synthesis of susceptible bacteria, is produced by an actinomycete. The main activity of this drug is to inhibit peptidoglycan synthesis, but it also has an effect on other aspects of bacterial metabolism. Vancomycin is a bactericidal antibiotic.

321.

C. Metronidazole, a nitroimidazole derivative, is active against most of the clinically significant

anaerobes. Only some of the non-spore-forming, gram-positive anaerobic bacilli and gram-positive anaerobic cocci are resistant to this agent. This drug acts to disrupt bacterial DNA through the production of cytotoxic intermediates.

322.

A. Gentamicin is a member of the aminoglycoside group of antibiotics. These drugs act on the 30S ribosomal subunit to inhibit protein synthesis. Gentamicin is particularly effective against a wide variety of gram-negative bacilli.

323.

B. Bone marrow toxicity is the major complication of chloramphenicol. Reversible bone marrow suppression with anemia, leukopenia, and thrombocytopenia occurs as a direct result of the agent on hematopoiesis. The second form of bone marrow toxicity is a rare but usually fatal aplastic anemia. The mechanism of this response is not known.

324.

A. Standard quality control strains maintained by the American Type Culture Collection (ATCC) should be tested routinely as recommended by the Clinical and Laboratory Standards Institute. Guidelines developed for the quality assurance of the disk-diffusion antimicrobial susceptibility test procedure recommended that the following organisms be used for this purpose: *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), and *Enterococcus fecalis* (ATCC 29212). Cultures of these organisms should be frozen or lyophilized to maintain their antimicrobial susceptibility pattern. Testing should not be done from stored cultures but rather from freshly grown 18- to 24-hour cultures.

325.

C. The chromogenic cephalosporin method is the most sensitive test for detecting the production of beta-lactamase enzymes. This yellow compound will become red if the organism produces the enzyme that breaks the beta-lactam ring. Nitrocefin, the commonly used compound, has a high affinity for most bacterial beta-lactamases.

Procedures and Biochemical Identification of Bacteria

326.

C. Normal flora can offer the host protection against infections by providing competition to pathogenic bacteria. The lungs are not typically colonized with bacterial flora. When diagnosing lower respiratory track infections, procedures such as bronchoscopy or percutaneous transtracheal aspirate are used to obtain a specimen that is not contaminated by upper respiratory tract flora.

327.

B. The flora of the female genital tract changes with age and the associated effects of pH and estrogen concentration in the mucosa. *Lactobacillus* spp. are the predominant flora during childbearing years. Earlier and later in life, staphylococci and corynebacteria predominate.

328.

C. The MIDI system is based on the analysis of fatty acids in the cell wall of microorganisms. The bacteria are grown under standardized conditions, and the fatty acids are extracted. The *Mycobacterium* fatty acids are analyzed by high-performance liquid chromatography. Gas liquid chromatography is used for other bacteria and yeasts. Results are compared to a computerized database.

329.

D. Most commercially available blood culture media contain the anticoagulant sodium polyanetholsulfonate (SPS). Anticoagulation is important because certain bacteria do not survive well within clotted blood. Within the clot, neutrophils and macrophages remain active and phagocytosis can occur.

330.

B. Incubation of inoculated bacteriologic culture media requires that attention be given to optimal temperature ranges, adequate moisture, and proper atmospheric conditions for growth. The optimal atmosphere for many clinically significant isolates is one that contains 5–10% carbon dioxide. Capnophilic environments may be obtained by using incubators equipped with a tank of carbon dioxide and a regulator. Candle jars produce only about a 3% concentration of carbon dioxide. The portable Fyrite carbon dioxide gas analyzer may be used for the daily monitoring of capnophilic incubators.

331.

B. Microbiologic examination of body fluids is less effective when bacteria become trapped in clotted specimens. The most effective anticoagulant for use in the microbiology laboratory is sodium polyanetholsulfonate (SPS) in a concentration of 0.025–0.05%. Fluids known to clot on standing should be transported to the laboratory in a sterile tube containing SPS. This polyanionic anticoagulant is also anticomplementary and antiphagocytic.

332.

B. One of the metabolites in the tricarboxylic acid cycle, citrate can serve as an energy source for some bacteria. The assessment of the ability of an organism to use citrate as its sole carbon source aids in the identification of the family *Enterobacteriaceae*. *Klebsiella pneumoniae* is able to use citrate with the production of alkaline by-products. A blue color and/or growth of the isolate on the streak line or both are indicative of a positive reaction. *Escherichia coli* is citrate negative.

333.

A. Infectious aerosols put laboratory professionals at risk for acquiring many diseases. The handling of clinical specimens that require pipetting, centrifugation, or decanting may produce infectious aerosols. Bacteria frequently are present in greater numbers in aerosol droplets than in the liquid medium.

334.

A. The deamination of the amino acid phenylalanine results in the formation of phenylpyruvic acid. Detection of the activity of this deaminase enzyme is accomplished by adding a 10% solution of ferric chloride to the growth on an overnight agar culture. Formation of a green color in the liquid on the agar slant indicates the presence of phenylpyruvic acid.

335.

C. Mannitol salt agar is highly selective and differential. It is used for the isolation and identification of staphylococcal species. The 7.5% concentration of sodium chloride results in inhibition of most bacteria other than staphylococci. Mannitol fermentation, as indicated by a change in the phenol red indicator, aids in the differentiation of staphylococcal species because most *S. aureus* isolates ferment mannitol (changing the color of the medium to yellow) and most

coagulase-negative staphylococci are unable to ferment mannitol.

336.

B. When inoculated on a sulfur-containing medium, organisms that produce hydrogen sulfide will demonstrate a partial blackening of a strip impregnated with a 5% solution of lead acetate. The strip is inserted above the medium in the tube and is secured under the closure, which seals the tube. The presence of liberated, dissolved sulfide gas reacts with the lead on the strip, and a black (lead sulfide) color develops. This test is useful for the detection of weak hydrogen sulfide-producing organisms, because the triple sugar iron agar is not a sensitive indicator.

337.

D. Nitrate reduction is a general characteristic of members of the family *Enterobacteriaceae*. An organism with this ability reduces nitrates, and nitrites are produced in the medium. After incubation, α -naphthylamine and sulfanilic acid are added to the medium. The presence of nitrites is indicated by the production of a red color within 30 seconds. If the reduction has gone on to nitrogen gas, the color change will not occur. The addition of zinc dust with no resulting color change confirms the reduction of nitrate to nitrogen gas.

338.

A. The decarboxylase activities of members of the family *Enterobacteriaceae* are important tests for their identification. When a decarboxylase broth is inoculated with a test organism, the organism first ferments the glucose present, which produces a color change from purple to yellow. The yellow color indicates acid production. An organism that possesses decarboxylase activity will then be able to attack the amino acid present, producing alkaline amines. The lowered pH (acid) activates the decarboxylase enzyme. The amines in turn raise the pH, and a color change from yellow to dark purple results.

339.

B. Oxidative-fermentative (OF) medium was first devised by Hugh and Leifson in an attempt to detect weak acid production from nonfermentative bacilli. By decreasing the amount of peptone (0.2%) used in conventional media, the formation of oxidative products from amino acids, which may neutralize the weak acids produced by the organism, is reduced, and the metabolic reaction can be demonstrated. Demonstration is further facilitated by an increase in the concentration of carbohydrate (1.0%) in the medium, along with a semisolid consistency. Bromthymol blue is the pH indicator.

340.

D. Glucose metabolism by certain organisms produces acetyl methyl carbinol (acetoin) as the chief end product. To demonstrate this reaction, 40% potassium hydroxide is added, which oxidizes acetoin to diacetyl. The prior addition of α -naphthol, which acts as a catalyst, produces a red color complex if acetoin is oxidized. Members of the genera *Klebsiella*, *Enterobacter*, *Hafnia*, and *Serratia* produce a positive reaction in this test.

341.

D. Blood cultures are one of the most important specimen types sent for bacteriologic examination. Knowledge of the various clinical conditions that produce bacteremia is essential for optimal recovery of the causative agent. Not all conditions produce continuous bacteremia, and organisms may be present in low numbers. If possible, cultures should be drawn before antimicrobial therapy is initiated and optimally before a fever spike. There is a period of 1–2 hours from the time of the release of bacteria into the bloodstream and the subsequent physiologic chill response. Between 10 and 20 mL of blood is recommended. Blood cultures should be held in the laboratory at least 1 week before being reported as negative.

342.

D. Bacterial metabolism of carbohydrates in the culture media produces the by-product carbon dioxide, which is captured as head gas in sealed culture vials. Manometric systems measure the head space pressure. It is possible to detect bacterial metabolism in these systems within only a few hours of inoculation.

343.

A. The acridine orange stain is used to detect low numbers of microorganisms in fluid and exudate samples. The application of this fluorescent dye enables the microbiologist to screen samples at low-power microscopic magnification. This technique is recommended for the routine screening of blood cultures and cerebrospinal fluid sediment smears because of its superior sensitivity as compared to the Gram stain.

344.

A. In the catalase test, hydrogen peroxide is reduced to water and oxygen. The formation of oxygen produces the bubbles seen in a positive test. The catalase test is used to differentiate the staphylococci (positive) from the streptococci (negative).

345.

B. Pyrrolidonyl- α -naphthylamide (PYR) is the substrate. The test detects the presence of the enzyme L-pyrrolidonyl arylamidase. The PYR test helps differentiate *Staphylococcus aureus* (positive) from *S. lugdunensis* (negative).

Case Studies

346.

C. Disseminated gonococcal infection produces symptoms of arthritis, especially in the major joints of the body. Samples of joint fluid from these patients should be inoculated to a selective medium for the isolation of *Neisseria gonorrhoeae* in addition to nonselective media. Thayer-Martin agar has a chocolate agar base formulated to support the growth of fastidious species of *Neisseria* while suppressing the growth of normal or indigenous flora by the addition of antimicrobial agents. Only about 50% of patients with gonococcal arthritis will have positive synovial fluid cultures.

347.

A. The anatomy of the female urethra allows bacteria from the perirectal region to reach the bladder easily. *E. coli* is the most common pathogen in uncomplicated community-acquired urinary tract infections. Other organisms are more prevalent in nosocomial or recurrent infections.

348.

A. *Salmonella* Typhi, the causative agent of typhoid fever, is commonly associated with invasion of the bloodstream. The presence of organisms is the result of an extravascular site of infection. The extravascular sites in the case of typhoid fever are the small intestine, the regional lymph nodes of the intestine, and the reticuloendothelial system. The bacteremic phase is seen before the organism can be recovered in stool.

349.

C. *Vibrio vulnificus* is responsible for septicemia after consumption of contaminated raw oysters. Infections are most severe in patients with hepatic disease, hematopoietic disease, or chronic renal failure and those receiving immunosuppressive drugs. Mortality in patients

with septicemia can be as high as 50% unless antimicrobial therapy is started rapidly.

350.

D. *Neisseria meningitidis* is a leading cause of bacterial meningitis. Disease is transmitted by respiratory droplets among people in prolonged close contact, such as in daycare centers. Chemoprophylaxis with rifampin is appropriate for those in close contact with the patient: household members, daycare staff, and classmates.

351.

C. *Arcanobacterium pyogenes* has been reclassified several times. It was formerly a member of the genera *Corynebacterium* and *Actinomyces*. *A. pyogenes* is a well-known animal pathogen causing soft tissue infections in a wide variety of farm animals. Mode of transmission to humans is unknown, but most cases occur in a rural environment and include a history of abrasion or undetected wounds with animal exposure. *Listeria monocytogenes* is also a gram-positive bacillus that is CAMP positive; however, it is catalase positive.

352.

D. *Staphylococcus aureus* has been isolated from a majority of the reported cases of the clinical syndrome described—toxic shock syndrome. First reported in the late 1970s, the disease was linked to the use of a specific brand of tampons. Symptoms are associated with the production of a pyrogenic exotoxin (toxic shock syndrome toxin-1; TSST-1) by the coagulase-positive *Staphylococcus aureus*.

353.

D. The etiologic agent in this case is *Yersinia enterocolitica*. Disease caused by this organism frequently mimics the symptoms of appendicitis, although it has been implicated in a variety of clinical illnesses such as bacteremia, cholecystitis, and mesenteric lymphadenitis. *Y. enterocolitica* grows slowly at 35°C and, unless in large numbers or pure culture, may be overlooked in the laboratory. A key finding for *Y. enterocolitica* is a positive urease.

354.

A. Lyme disease is an inflammatory disease seen predominantly in the northeast and mid-Atlantic U.S. during the summer months. The initial symptoms of this disease may be followed months later by more serious complications, such as meningoenitis, myocarditis, and arthritis of the large joints. The etiologic agent of this tick-borne disease is *Borrelia burgdorferi*. The spirochetes causing Lyme disease have not been demonstrated in peripheral blood smears. An indirect immunofluorescence test and an ELISA test are available for the detection of specific antibody in the patient's serum. The western blot assay is often used for serologic confirmation.

355.

D. Human infections caused by *Leptospira* characteristically produce the clinical symptoms of fever, anemia, and jaundice. Weil disease is another name for leptospirosis. Infections result from contact with the urine or tissue of infected animals like rats and mice or from water contaminated with urine of these animals. Most infections resolve in about a week, but they can go on for much longer and can cause fatal kidney and liver damage.

356.

D. *Yersinia enterocolitica* causes a variety of infections. This organism is able to grow at

refrigerator temperatures (4°C). Contamination of stored blood units is not visually detected because the organism is able to reproduce in red blood cells without causing lysis or a color change.

357.

A. *Mycobacterium marinum* produces lesions on the skin or the extremities of humans. This species of *Mycobacterium* is a free-living organism found in salt or brackish water. Human infection characteristically follows trauma to the body in or around water.

358.

B. *Listeria monocytogenes* has been associated with human disease following the ingestion of unpasteurized dairy products. The organism is capable of replicating at refrigerator temperatures and is commonly found in low numbers in animal products. Listeriosis associated with contaminated food, in uncompromised patients, usually produces a self-limiting, nonspecific febrile illness.

359.

A. *Escherichia coli* 0157:H7 is associated with hemolytic uremic syndrome. These strains produce verotoxin and are associated with outbreaks of diarrheal disease following ingestion of undercooked hamburger at fast-food restaurants and contact with calves at petting zoos. Cattle infected with this strain serve as the reservoir, and humans become infected by eating products made from their meat or contaminated with their excretions.

360.

C. The clinical presentation suggests the etiologic agent is *Legionella pneumophila*. The Gram stain is not helpful in making the diagnosis because of the poor staining quality of this microorganism. Examination of the sputum using fluorescent antibody to *L. pneumophila* could provide a rapid positive identification.

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